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ABSORPTION PHENOMENA IN A CONDENSED SPARK SOURCE[1]

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Abstract

Intensity ratios of certain line pairs in the spectrum of magnesium, of mercury, and of tin have been found to depend strongly on the amount of the element introduced into the source, even when the amount is within the range dealt with in making "trace" analyses. Line pairs involving transitions to a level in the ground term and to a considerably higher level exhibited this behaviour. It is attributed to the occurrence of partial absorption, mainly within the limits of the discharge. The dependence of the intensity ratios on the amount of the element present was not affected appreciably by air currents of 80 cm. per sec. velocity; it was affected however by currents of 1500 cm. per sec. velocity. The data lead to the conclusion that the absorption effects are normally quite stable, and are unlikely to introduce serious errors in analytic determinations.

Although considerable information, both experimental and theoretical, is available concerning the absorption of radiation in passing through layers of vaporized material (see, for example, (10)), little is known of the details of absorption in the arc or spark at atmospheric pressure. This is particularly true of sources used under conditions normally encountered in spectrographic analysis. It appeared desirable to investigate absorption phenomena for radiation from elements present in the source in extremely small amounts, and particularly to ascertain whether they are subject to fluctuations capable of introducing serious errors in analytic determinations.

In general, radiation involving a transition to a level in the ground term of an atom may be expected to exhibit self-absorption after passing through layers of the discharge, through a relatively cold cloud of vapour about the discharge, or through both. Such radiation is usually associated with spectral lines selected for measurement in making "trace" analyses, i.e., sensitive lines. On the other hand, radiation associated with higher final levels may be expected to suffer much less absorption for two reasons, (*a*) the populations of the higher atomic levels are small compared to that of the ground state in the discharge itself, and (*b*) cold vapour surrounding the discharge is highly transparent to the radiation. On this basis the intensity ratios of suitably chosen line pairs should depend on the amount of the element present in the source; the dependence should disappear when absorption effects become negligible.

This article describes a study of the dependence of selected intensity ratios in the spectrum of magnesium, of mercury, and of tin, on the amount of the element present on the source electrode. A common type of condensed spark source was used. The investigated range extended from 4×10^{-7} to 8×10^{-4} mg. per mm.^{2} (of electrode surface) for magnesium, from 2×10^{-5} to 8×10^{-4} mg. per mm.^{2} for mercury, and from 8×10^{-6} to 8×10^{-4} mg. per mm.^{2} for tin. In the lower limit the deposit on the electrode approached the least quantity detectable. In addition, experiments were performed with an air blast directed against the source, in an attempt to influence the absorption phenomena. Air currents of 80 cm. per sec. velocity had little or no effect, but currents of 1500 cm. per sec. velocity influenced the dependence of intensity ratios on the amount of the element present.

1. Experimental

The condensed spark source in which the samples were excited was similar to that used by Langstroth and Newbound (8). The primary voltage of the power transformer was controlled by an autotransformer; in operation it was adjusted to the minimum value consistent with the passage of a single spark across the gap on every half cycle of the line voltage. Copper electrodes of the point-movable-plane type (2) were used. The area of the plane electrode was 1.0×1.3 cm.^{2}, and the electrode separation was 2.5 mm.

Spectra were photographed with a medium quartz spectrograph constructed at the University (slit width used, 60 microns). In order that intensity ratios of lines differing considerably in intensity might be determined from a single spectrum, a narrow antimony–quartz weakener (6) was placed over the central portion of the spectrograph slit. As a result, each spectral line on the plate exhibited a central section of decreased density in which photometric measurement on strong lines was possible. A magnified image of the discharge was formed in the plane of the slit with a quartz–fluorite lens. The intensity of light falling on the slit was adjusted to the desired value for each set of conditions by inserting an appropriate screen weakener immediately behind the condensing lens.

In order to study the dependence of the relative intensities of selected magnesium lines on the amount of magnesium present on the electrode, a graded series of aqueous solutions of magnesium chloride hexahydrate was prepared. Each solution contained 40 mg. per cc. of potassium acetate to act as a "buffer". The magnesium content ranged from 0.0010 mg. per cc. to 2.0 mg. per cc. Since 0.050 cc. of each solution was placed on a plane electrode and dried under reduced pressure, the magnesium content of the deposits ranged from 4×10^{-7} to 8×10^{-4} mg. per mm.^{2} of electrode surface.

Each of the prepared electrodes was sparked for 30 sec., and the spectrum was photographed. During the exposure the plane electrode was so moved that the base of the discharge passed once over the electrode surface, continually advancing to a fresh deposit. Use of the appropriate screen weakenei

for each sample, together with the antimony weakener, permitted the photo-
graphic density of the selected lines to be kept on the normal exposure portion
of the characteristic curve of the plate. Calibration spectra were photo-
graphed on each plate with the aid of a brass arc operated at constant current
and arc length, and a rotating step-sector (eight steps).

A similar procedure was adopted in the investigation of intensities in the
mercury and in the tin spectrum. The amount of mercury in the electrode
deposit was varied from 2×10^{-5} mg. per mm.^{2} to 8×10^{-4} mg. per mm.^{2},
and of tin from 8×10^{-6} mg. per mm.^{2} to 8×10^{-4} mg. per mm.^{2}

Intensity measurements were made by means of a microphotometric
method (for details see (9)). Since the relative intensities for a particular
plate were determined in terms of intensities of various lines in the calibration
spectra for that plate, comparison with the results for another plate was made
through spectra of samples common to both. By overlapping the concen-
tration ranges in series of samples taken on different plates it was possible to
express all results in the same arbitrary units. Intensities of strong lines were
determined from measurements on the central "weakened" portion of a
spectrum; intensities of the fainter lines were determined from measurements
on the "unweakened" portions on either side, and the mean value was taken.
Background corrections were made when necessary by determining the
intensity of the background in the vicinity of the line, and subtracting it from
the measured "line plus background" intensity. The magnitude of this cor-
rection increased with decreasing concentration of the element in the sample
because of the progressively greater transmission required for the screen
weakener. For this reason, the results obtained with very low concentrations
are less accurate. It is believed that the intensity ratios are in error by less
than 7%, except for the lowest concentrations where there may be errors of
10%.

To study the effect of an air blast on the absorption phenomena, the experi-
ments were repeated with an air jet directed against the source. The air
passed through a dust filter, a needle valve, and finally through a Pyrex tube
placed horizontally and at right angles to the optic axis of the collimator.
With this arrangement, light from the luminous tail which appeared on the
discharge did not enter the spectrograph slit. The air velocity was estimated
from subsidiary experiments by observation of the rate of displacement of
water in a large container. Experiments were performed with air velocities
of 80 cm. per sec., and 1500 cm. per sec.

The origin of the magnesium, mercury, and tin lines studied is indicated in
the partial energy level diagrams of Figs. 1 and 2 (1, 3, 4, 5). Several of
the lines measured were blends, owing in part to the low resolution of the
spectrograph used with a relatively wide slit. It is believed that this circum-
stance does not detract seriously from the results. The blends were:

Mg 2937 Å. This was a blend of Mg I 2936.895 Å and Mg II 2936.537 Å.
The intensity of the latter is estimated to be slightly the greater (4).
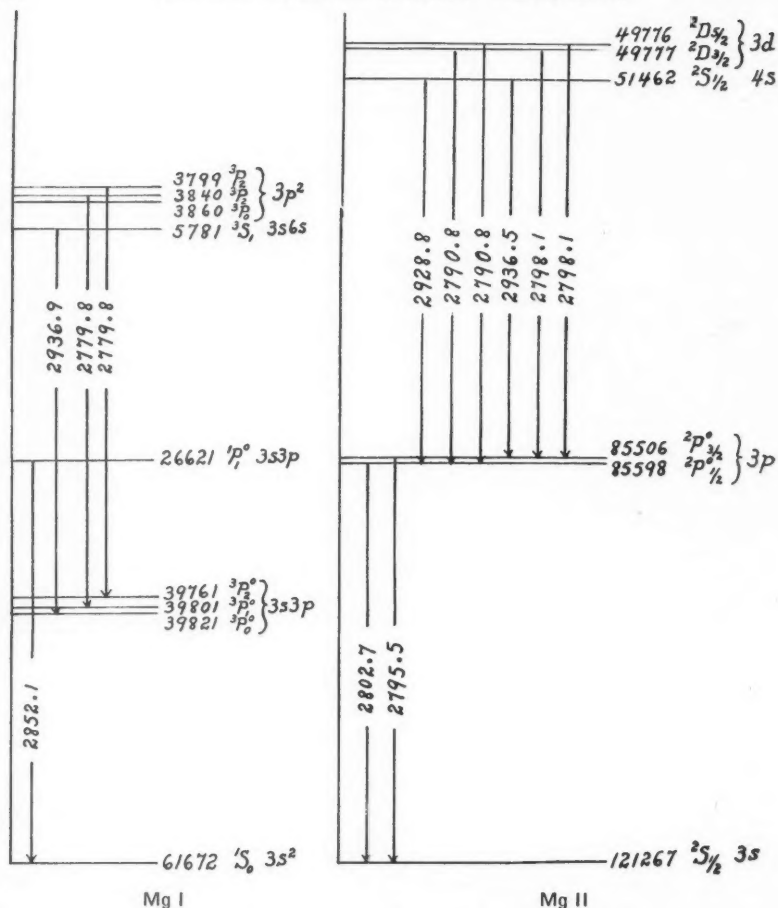
FIG. 1. *Partial energy level diagram for magnesium.*

Both lines however involve final levels far above the ground level of the atom and ion (21851 and 35761 cm.$^{-1}$ respectively).

Mg I 2780 Å. This was a blend of $3s3p\ ^3P_1^0 - 3p^2\ ^3P_1$ and $3s3p\ ^3P_2^0 - 3p^2\ ^3P_2$, both having a wave-length of 2779.834 Å. Both initial and final levels lie close together (separations 40.6 and 40.9 cm.$^{-1}$ respectively).

Mg II 2791 Å. This was a blend of $3p\ ^2P_{1/2}^0 - 3d\ ^2D_{5/2}$ and $3p\ ^2P_{1/2}^0 - 3d\ ^2D_{3/2}$, both having a wave-length of 2790.787 Å. They have a common final level and the initial level separation is small.

Mg II 2798 Å. This was a blend of $3p\ ^2P_{3/2}^0 - 3d\ ^2D_{5/2}$ and $3p\ ^2P_{3/2}^0 - 3d\ ^2D_{3/2}$, both having a wave-length of 2798.06 Å. They have a common final level and the initial level separation is small.

Hg I levels:
12750 $^3D_3$
12785 $^3D_2$
12845 $^3D_1$ } 6s6d
12848 $^1D_2$

Transitions: 2967.5, 2967.3, 3131.8, 3131.5, 3650.1

40138 $^3P_2^o$
44769 $^3P_1^o$ } 6s6p
46536 $^3P_0^o$

2536.5

84179 $^1S_0$ 6s^2

Hg I

Sn I levels:
15509 $^1I_2^o$ 5p($^2P_{1/2}$)5d

19935 $^1P_1^o$ } 5p($^2P_{1/2}$)6s
20563 $^3P_2^o$

24278 $^3P_1^o$ } 5p($^2P_{1/2}$)6s
24551 $^3P_0^o$

Transitions: 2863.3, 3034.1, 3009.1, 2840.0, 3262.3, 2860.6

50579 $^1D_2$
55764 $^3P_2$ } 5p^2
57500 $^3P_1$
59192 $^3P_0$

Sn I
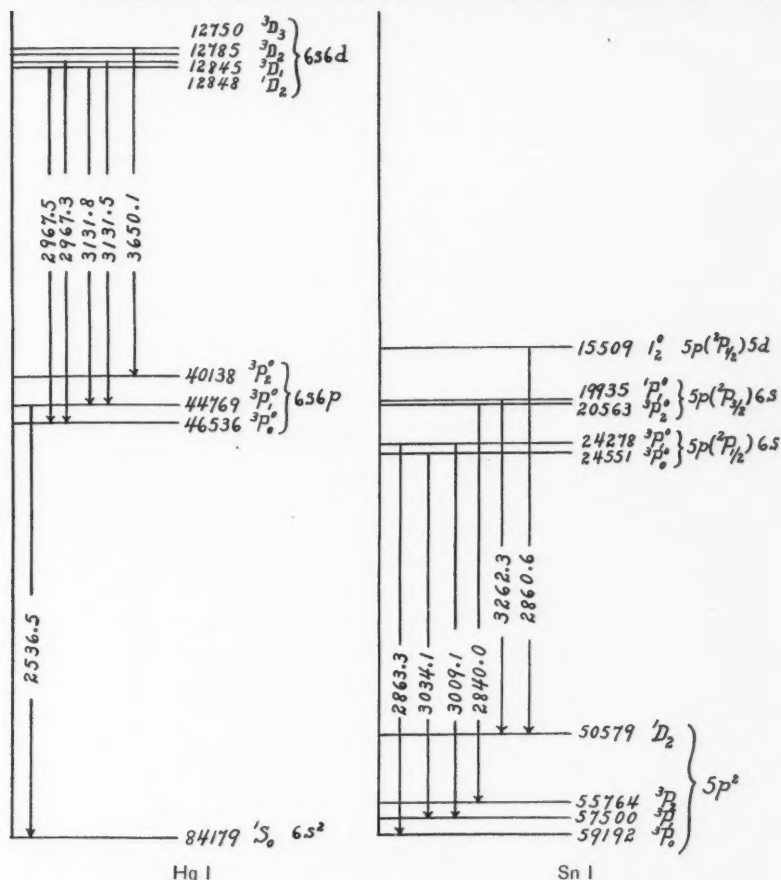
FIG. 2. *Partial energy level diagrams for mercury and tin.*

Hg I 2967 Å.   This was a blend of 2967.278 Å, 2967.517 Å, and 2967.592 Å, with estimated intensities (4) respectively 100, 5, and 10. The first two have a common final level and small initial level separation; the classification of the last, which contributes only a small part to the combined intensity, is unknown to the writers.

Hg I 3132 Å.   This was a blend of 3131.546 Å and 3131.833 Å. These lines have a common final level and the initial level separation is small.

2. Results

(a) Results obtained in still air.   Data describing the dependence of the intensity ratios on the amount of the element present on the electrode are given in Tables I (Mg), II (Hg), and III (Sn).   The results for each element

represent a separate series of experiments. The ratios for the greatest deposit of the element have been set equal to 100. In all ratios the numerator is the line having the higher final level. The line pairs are arranged to involve progressively higher atomic levels as one proceeds toward the right-hand side of the tables.

TABLE I

DEPENDENCE OF INTENSITY RATIOS OF Mg I AND Mg II LINE PAIRS ON THE AMOUNT OF MAGNESIUM PRESENT ON THE ELECTRODE. THE DATA WERE OBTAINED IN STILL AIR

Mg on the electrode, mg. per mm.² (× 10⁶)	Mg I			Mg II							
	$\frac{2937}{2852}$	$\frac{2780}{2852}$	$\frac{2780}{2937}$	$\frac{2796}{2803}$	$\frac{2929}{2803}$	$\frac{2791}{2803}$	$\frac{2798}{2803}$	$\frac{2929}{2796}$	$\frac{2791}{2796}$	$\frac{2798}{2796}$	$\frac{2791}{2798}$
800	100	100	100	100	100	100	100	100	100	100	100
400	68	75	113	106	82	76	76	79	73	72	100
200	50	66	130	—	62	62	64	—	—	—	97
80	34	44	150	105	52	47	54	51	45	40	108
40	33	38	120	114	42	39	42	40	37	33	114
8	15	21	140	140	35	29	33	26	22	24	112
5.3	—	—	—	140	32	29	26	24	21	23	108
4.0	12	14	111	140	30	26	23	22	19	20	112
2.7	9.5	11	104	130	32	24	23	24	18	17	109
0.8	9.4	9.3	—	140	—	25	18	—	17	13	130
0.4	—	—	—	140	—	25	20	—	19	13	—

TABLE II

DEPENDENCE OF INTENSITY RATIOS OF Hg I LINE PAIRS ON THE AMOUNT OF MERCURY PRESENT ON THE ELECTRODE. THE DATA WERE OBTAINED IN STILL AIR

Hg on the electrode, mg. per mm.² (× 10⁶)	Hg I				
	$\frac{2967}{2537}$	$\frac{3132}{2537}$	$\frac{3650}{2537}$	$\frac{3132}{2967}$	$\frac{3650}{2967}$
800	100	100	100	100	100
400	64	56	63	88	99
200	27	27	29	101	108
80	19	18	20	98	106
40	15	17	16	117	106
20	16	15	14	93	84

(b) *Results obtained with an air blast.* Results obtained with air blasts of 80 cm. per sec. and 1500 cm. per sec. are shown in Figs. 3, 4, and 5, where they are compared with the results obtained in still air.

3. Discussion

The data of Tables I, II, and III show a marked dependence of the intensity ratios of certain line pairs on the amount of the element present on the electrode. For example, the ratio for Mg I 2780/2852 increased by a factor

TABLE III

DEPENDENCE OF INTENSITY RATIOS OF Sn I LINE PAIRS ON THE AMOUNT OF TIN PRESENT ON THE ELECTRODE. THE DATA WERE OBTAINED IN STILL AIR

Sn on the electrode, mg. per mm.² ($\times 10^6$)	Sn I					
	$\dfrac{3034}{2863}$	$\dfrac{3009}{2863}$	$\dfrac{2850}{2863}$	$\dfrac{3262^*}{2863}$	$\dfrac{3009}{3034}$	$\dfrac{2850}{3262^*}$
800	100	100	100	100	100	100
400	100	94	85	98	92	87
200	104	90	66	93	85	71
80	97	84	59	87	84	68
20	100	82	55	82	81	66
8	105	91	51	76	88	67

There may have been a systematically varying error in the measurement of the intensity of his line, as pointed out in the discussion.
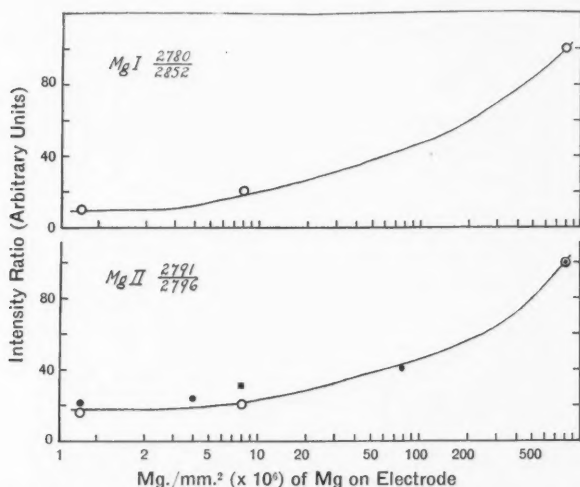

FIG. 3. *The effect of an air blast on the curves for intensity ratios vs. amount of magnesium on the electrode. The solid lines represent the data obtained in the absence of an air blast; individual values, which are given in Table I, are not indicated on the graph. Values obtained with an air current of 80 cm. per sec. velocity are indicated by ○; those obtained with a current of 1500 cm. per sec. velocity are indicated by ●.*

of 11 when the amount of magnesium on the electrode was increased from 8×10^{-7} mg. per mm.² to 8×10^{-4} mg. per mm.² On the other hand, certain other line pairs showed no evidence of such behaviour. Line pairs having one final level in the ground term and the other considerably above it belong without exception to the former class; those involving close lying final levels, whether these levels be in the ground term or not, belong to the latter class. An apparent exception occurs for the Sn I line pair 2850/3262. These lines

FIG. 4.   *The effect of an air blast on the curves for intensity ratios vs. amount of mercury on the electrode.   As in Fig. 1, the solid line represents the data obtained in the absence of an air blast.   Intensity ratios obtained with an air current of 80 cm. per sec. are indicated by $\bigcirc$; ratios obtained with a current of 1500 cm. per sec. are indicated by $\bullet$.*
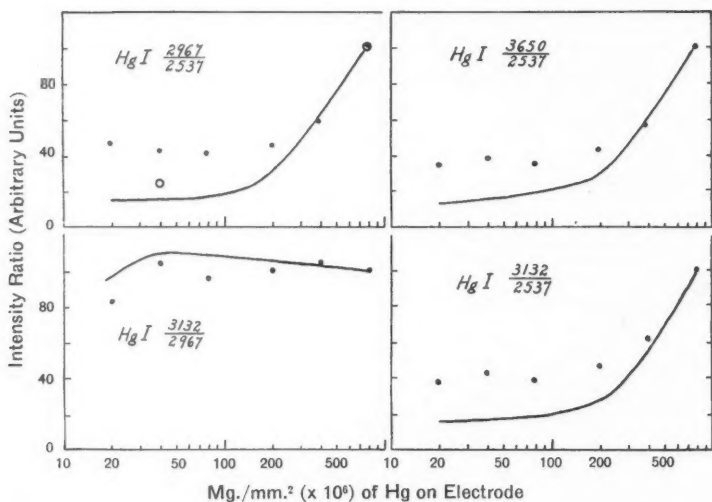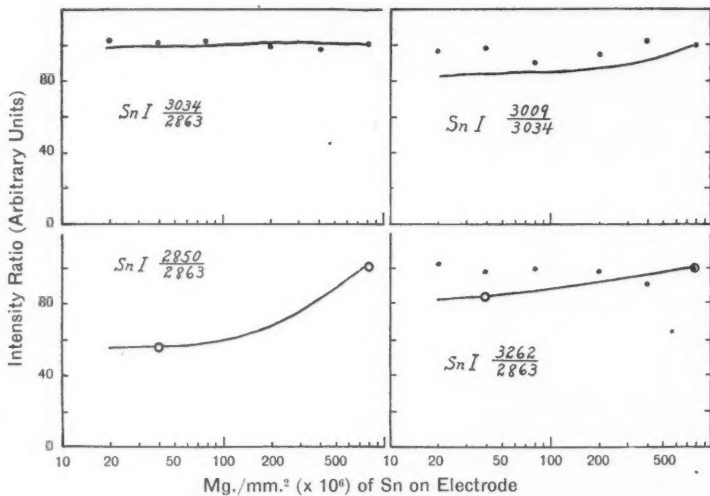

FIG. 5.   *The effect of an air blast on the curves for intensity ratios vs. amount of tin on the electrode.   The solid line represents the data obtained in the absence of an air blast, and $\bigcirc$ and $\bullet$ represent intensity ratios obtained with air currents of 80 cm. per sec. and 1500 cm. per sec. respectively.*

have a common final level, yet their intensity ratio decreased with decreasing amount of tin on the electrode.   The initial levels are separated by 4426 cm.$^{-1}$. The observed decrease may be spurious, for the following reason.   The line 3262 Å lies between the copper lines at 3247 Å and 3274 Å—the strongest lines in the copper spectrum.   Hence the background intensity for 3262 Å was difficult to determine accurately.   As the amount of tin on the electrode was decreased, screen weakeners of progressively higher transmission were used, with the result that the photographic density of lines of the copper spectrum on the plate became great.   Underestimation of the background intensity for 3262 Å would produce data involving 3262 Å similar to those observed.

With the apparent exception just noted, the picture presented by the data is that to be expected from the occurrence of appreciable absorption.   The observed intensity changes in the spectra of Mg I, Mg II, and Hg I are similar, as might be expected from the similarity of the energy level diagrams.   They are probably almost entirely due to partial absorption of the 2852 Å, 2803 Å, 2796 Å, and 2537 Å radiation.   Partial absorption of lines having final levels considerably above the ground state must be relatively unimportant.   On the other hand, it is probable that all the investigated tin lines are affected to some extent by absorption, those ending on the $5p^2$ $^1D_2$ level being least influenced.   The basis for these expectations lies in a comparison of the estimated relative populations of various final levels for an assumed Boltzman distribution (see (7)) at a temperature of 6000° K. (Table IV).   The mean "effective" temperature of the absorbing region of vapour is probably no higher than 6000° K., if as high.

TABLE IV

ESTIMATED RELATIVE POPULATIONS OF FINAL LEVELS FOR A BOLTZMAN DISTRIBUTION AT 6000° K.

Element	Level	Relative population	Element	Level	Relative population
Mg (neutral atom)	$3s^2$ $^1S_0$	1000	Hg	$6s^2$ $^1S_0$	1000
	$3s3p$ $^3P_0^0$	5		$6s6p$ $^3P_0^0$	0.1
	$3s3p$ $^3P_1^0$	15		$6s6p$ $^3P_1^0$	0.2
	$3s3p$ $^3P_2^0$	25		$6s6p$ $^3P_2^0$	0.1
(ion)	$3s$ $^2S_{1/2}$	1000	Sn	$5p^2$ $^3P_0$	1000
	$3p$ $^2P_{1/2}^0$	0.2		$5p^2$ $^3P_1$	2000
	$3p$ $^2P_{3/2}^0$	0.3		$5p^2$ $^3P_2$	2200
				$5p^2$ $^1D_2$	650

Curves for intensity ratios of "variable" line pairs plotted on log-log paper against the amount of the element present on the electrode are concave upwards, the slope tending toward zero as the amount decreases.   They

indicate that in the region of 2×10^{-6} mg. per mm.^{2} of magnesium, and of 2×10^{-5} mg. per mm.^{2} of mercury, absorption effects become relatively unimportant, i.e., the ratios tend to reach a constant value. These amounts involve approximately equal numbers of atoms. They approach the least quantities of magnesium and mercury detectable with this source.

A simple calculation shows that the amount of an element (as vapour) effective in producing detectable absorption must be extremely minute. For example, from Table I, Mg I 2852 Å was markedly absorbed when the amount of the element on the electrode was as small as 10^{-5} mg. per mm.^{2}. Assuming one-quarter of the deposit to be removed in sparking for 30 sec. (a high upper limit), and to be distributed with spherical symmetry about the source, the average amount of magnesium present in the solid angle subtended by the condensing lens comes out to be about 6×10^{-7} mg. The data obtained with an air blast directed against the discharge give reason to believe that this figure is far too high.

Results partially presented in Figs. 3, 4, and 5 indicate that air currents of 80 cm. per sec. velocity had little or no effect on the absorption phenomena. Air currents of 1500 cm. per sec. velocity did reduce the absorption, but not to the extent that might be expected if the principal absorbing medium was a cloud of cool vapour collected about the source. Air velocities of 80 cm. per sec. would move such a cloud a distance of about 0.7 cm. in the interval between sparks (1/120 sec.), while air velocities of 1500 cm. per sec. would move it about 12 cm. The failure of the air blasts to produce more marked effects suggests that the absorption takes place largely within the limits of the discharge itself, and that for radiation from a single spark, it is caused mainly by material released during that spark and possibly a few preceding ones. On this basis, collection of cool vapours about the discharge plays a relatively minor role.

Because of the occurrence of absorption phenomena in the concentration range of interest in making trace analyses, the working curves for lines with high final levels may differ markedly in character from those for lines with low final levels, as may be inferred from the results of Tables I, II, and III. Providing the phenomena are not subject to appreciable fluctuations this is not a serious matter. The results discussed above show that the phenomena are remarkably "stable". They indicate that no appreciable errors are introduced in analytic determinations by variations in absorption due to varying air currents of velocities commonly encountered in the laboratory.

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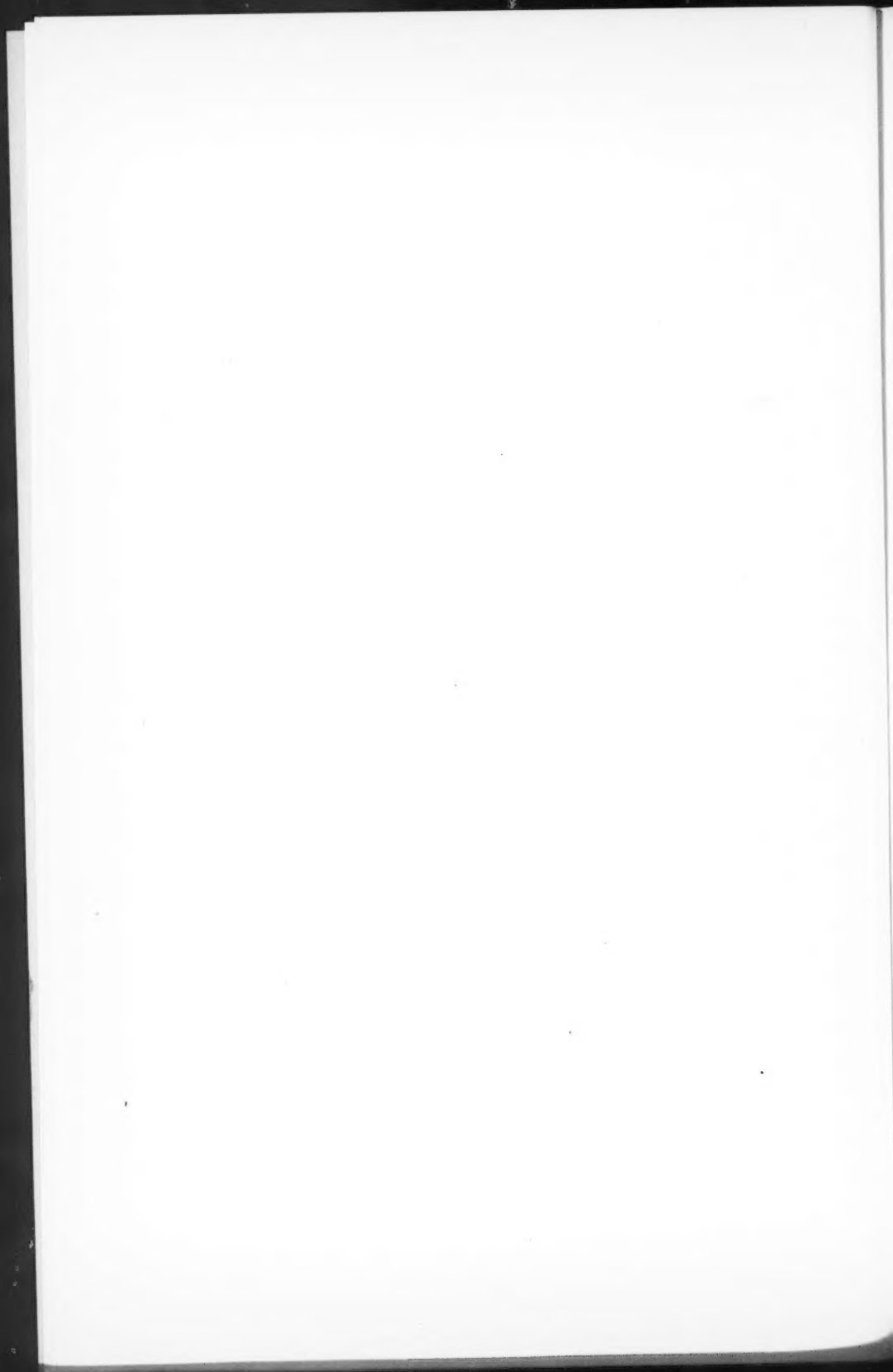
LES GLUCIDASES ET LES GLUCIDES D'*IPOMOEA BATATAS* ET DE *SOLANUM TUBEROSUM*[1]

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Résumé

L'action des jus dialysés d'*Ipomoea Batatas* et de *Solanum tuberosum* sur l'amidon produit du maltose, du sucrose, et du sucre interverti. Ces jus contiennent une maltogène-amylase, une sucrogène amylase, et l'invertase. La maltase est absente. Dans ces patates cependant on ne trouve pas de maltose mais du sucrose et du sucre interverti. L'hydrolyse enzymatique conduira à une nouvelle hypothèse de la constitution de l'amidon.

Introduction

La présence d'une amylase dans *Solanum tuberosum* est reconnue depuis les travaux de Windish et Jetter (12, 13). Gore a démontré que l'*Ipomoea Batatas* contient aussi une amylase (6).

Mais la reconnaissance d'autres glucidases et des produits formés par le travail de ces amylases n'amène pas toujours l'accord entre les chercheurs. Il suffit de rappeler les publications de Doby et Bodnar (4), Gore (7), Miyake (9) et Nelson et Auchincloss (10).

Comme l'activité de ces amylases est tantôt appréciée par la coloration avec l'iode, tantôt par la mesure du pouvoir réducteur, le plus souvent exprimé en maltose (5); cela explique déjà les résultats variés obtenus. Nous verrons qu'en plus des conditions variables, des facteurs nouveaux peuvent influencer ces résultats.

Hanes (8) montre aussi que l'amylase de la patate ordinaire est accompagnée d'une phosphorylase qui transforme l'amidon en glucose-1-phosphate lequel peut ensuite passer jusqu'aux hexose-6-phosphates réducteurs et fructose diphosphate (11). Nous avons été conduits à l'étude des glucidases et des glucides de la patate douce et de la patate ordinaire à la suite de la reconnaissance dans la sève d'érable, d'une amylase qui hydrolyse l'amidon en sucrose (2).

Partie expérimentale

Les solutions d'amidon sont préparées à partir d'amidon soluble; sa suspension dans l'eau froide est jetée dans un grand volume d'eau bouillante; après refroidissement, du toluène est additionné en excès.

Les tampons sont constitués de solutions $M/15$ de phosphate de sodium, de potassium, et d'acide phosphorique.

Le pouvoir réducteur des essais est déterminé par la méthode de Luff et Scholl (3, p. 830) et exprimé en milligrammes de glucose ou en millilitres de thiosulfate de sodium $N/20$.

La préparation des extraits

Des patates sucrées (*Ipomoea Batatas*) et des patates ordinaires (*Solanum tuberosum*) sont rapées, séchées à la température de la chambre, réduites en poudre suffisamment fine pour être tamisée à 40 mailles. Cent grammes de la poudre sont agités avec 500 ml. d'eau additionnée de toluène, puis macérés à la glacière pendant 24 hr. Après filtration sur toile, les jus (400 ml. de chacun) sont mis à dialyser dans des tubes de cellophane contre l'eau du robinet.

Signalons que le jus obtenu par pression des patates a un pH d'environ 5.0, tandis que celui que nous obtenons par macération de la poudre est de 6.5 environ.

Après une dialyse de deux jours, les jus sont concentrés à 400 ml. en maintenant les sacs dialyseurs à la température de la chambre, dans un courant d'air.

Le dépôt qui se forme au cours de la dialyse n'a pas été séparé, des essais nous ayant démontré qu'il entraînait des enzymes. Une forte agitation du jus précédait donc chaque prise d'échantillon.

Les amylases

Si l'on étudie à 20° C. l'action de 10 ml. de jus dialysé sur 10 ml. d'amidon à 1%, en présence de 10 ml. de tampon à des pH variant de 3.3 à 8.0, on constate que la réaction avec l'iode montre un maximum d'activité à pH 6.5 pour *Ipomoea* et à pH 5.5 pour *Solanum.*

Le pouvoir réducteur cependant indique un maximum d'activité qui se déplace dans les deux cas vers le pH 4.0 au cours de la digestion.

Cette variation du maximum d'activité en fonction du pH dépend aussi de la concentration des enzymes par rapport à l'amidon. Ces constatations dans les jus dialysés de *Solanum* et d'*Ipomoea* nous ont conduits à la recherche des autres glucidases qu'ils pouvaient contenir.

La sucrogène amylase

Après une digestion pendant 72 hr., à 20° C. d'un mélange de 10 ml. d'extrait dialysé, 20 ml. d'amidon à 1%, 10 ml. de tampon à pH 6.5, et 10 ml. d'eau, les ferments sont détruits par ébullition et le milieu est ensuite tamponné à pH 4.5.

Des aliquotes sont additionnées de 1 ml. d'une solution d'invertase; tandis que d'autres reçoivent la même quantité d'invertase bouillie.

Après 12 hr., la différence du pouvoir réducteur exprimé en glucose entre ces deux groupes d'aliquotes est de 4 mg.

Ces essais avec le jus dialysé d'*Ipomoea* et de *Solanum* ont été répétés en remplaçant l'amidon à 1% par une solution dialysée d'amidon à 2%. Les résultats sont analogues. Ils indiquent la formation de sucrose.

L'invertase

Des échantillons de 10 ml. d'une solution de sucrose à 1% et de 10 ml. de tampon à pH 4.5 sont additionnés soit de 10 ml. d'extraits dialysés de *Solanum* et d'*Ipomoea* d'une part, soit de la même quantité des mêmes extraits mais bouillis d'autre part. Après 24 hr. à 20° C., la différence du pouvoir réducteur entre les prises bouillies et non bouillies indique une hydrolyse qui va jusqu'à 75% du sucrose.

La maltase

En plus de la recherche de cette enzyme dans les jus dialysés et tamponnés à pH 6.7 et qui fut négative, nous avons procédé aux expériences suivantes avec des jus obtenus en broyant les patates en présence de toluène et pressant ensuite le tout à travers une toile. Ces jus ont un pH de 4.7 à 4.8.

Nous rapportons l'expérience faite à 25° C. avec *Solanum tuberosum*. Les résultats sont les mêmes avec *Ipomoea Batatas*.

Quatre échantillons de jus frais ont été distribués dans des ballons jaugés de 200 ml.

N° 1: 50 ml. de jus non chauffé et eau diluée à 200 ml.

N° 2: 50 ml. de jus bouilli et eau diluée à 200 ml.

N° 3: 50 ml. de jus bouilli et 100 ml. de maltose (1 mg.) à 200 ml.

N° 4: 50 ml. de jus non chauffé et 100 ml. de maltose (1 mg.) à 200 ml.

Au temps zéro, 25 ml. de chaque échantillon sont pipettés; les N°s 1 et 4 sont inactivés par ébullition; tous sont ensuite étendus à 50 ml. Le pouvoir réducteur est déterminé sur 25 ml. et exprimé en millilitres de la solution de thiosulphate $N/20$ nécessaire à la titration en retour. (Méthode Luff et Scholl.)

La même manipulation se répète après 48 hr.: voir le Tableau I.

TABLEAU I

N°	Ml. de $Na_2S_2O_3$, $N/20$	
	Au début	Après 48 hr.
1	19.0	15.6
2	19.0	18.8
3	16.4	16.4
4	16.2	13.0

La différence après 48 hr. entre le N° 2 et le N° 1 est la même qu'entre N° 3 et N° 4; et aussi la même qu'entre N° 4 au début et N° 4 après 48 hr., et qu'entre N° 1 au début et N° 1 après 48 hr.; soit 3.2 à 3.4 cc. Cette dif-

férence correspond à l'augmentation du pouvoir réducteur des glucides contenus dans les patates.

La différence au temps zéro, entre N° 2 et N° 3, se retrouve encore après 48 hr.: soit 2.4 à 2.6 cc., et correspond à la quantité de maltose ajoutée (6.25 mg.) dans les prises de dosage. *L'hydrolyse du maltose n'a donc pas lieu.*

Etude des osazones

Pour identifier les glucides des patates sucrées et ordinaires, de même que ceux fournis par l'action des amylases de ces patates sur l'amidon, nous nous sommes servis de la méthode de Fisher des osazones (3, p. 270): 1 partie de sucre, 2 parties de chlorhydrate de phénylhydrazine, 3 parties d'acétate de sodium dans 20 parties d'eau, au bain d'eau bouillante pendant 30 min.

Dans des essais préliminaires nous avons constaté que la forme cristalline des osazones était très variable; cela nous a conduit à faire une étude systématique de la glucosazone, de la maltosazone, et de la cellobiosazone que nous soupçonnions devoir rencontrer.

La glucosazone

Le glucose nous donne à chaud la glucosazone jaune-pâle de forme classique: aiguilles fines réunies en gerbe.   C'est celle obtenue directement; nous l'appellerons, glucosazone 1.

Par contre, si l'on filtre la préparation chaude, le filtrat nous donne déjà à 80° C. un précipité jaune-orange en petites boucles plates et courtes ou en forme d'étoile, et qui n'est plus soluble dans l'eau bouillante.

Il est à noter qu'on n'obtient cette forme cristalline qu'après filtration à chaud.   Si la préparation est refroidie sans filtration, on ne trouve pas ce produit que nous pouvons appeler: glucosazone 2.   Elle est soluble dans l'alcool chaud et garde en cristallisant dans ce solvant sa forme anormale; toutefois, après de nombreuses recristallisations, elle tend à revenir à la forme cristalline classique, et possède toujours le point de fusion de la glucosazone 1: au capillaire: 201 à 202° C., au bloc Maquenne: 228 à 230° C.

De plus si l'on ensemence une solution alcoolique saturée à chaud de glucosazone 2 avec de la glucosazone 1, la cristallisation tend vers la glucosazone 1. Dans les conditions ci-haut mentionnées, le lévulose et le sucrose donnent aussi ces deux formes cristallines d'osazones.

La maltosazone

Ce n'est qu'après refroidissement que nous avons un précipité cristallin abondant.   Les cristaux sont en forme d'oursin ou de pelote; ce qui n'est pas du tout la forme cristalline reconnue de la maltosazone.

Recristallisation

Après lavage à l'eau froide, une partie du précipité est dissoute dans l'alcool à 50% bouillant, et l'autre partie dans l'eau bouillante.   On constate qu'après deux recristallisations dans l'alcool, la maltosazone a des formes variables

qui se rapprochent plus ou moins de la forme cristalline normale généralement reconnue: baguettes plates et striées terminées en fer de lance.

La maltosazone dans l'eau prend et garde, dès la première recristallisation la forme cristalline normale de la maltosazone.

La cellobiosazone

Nous avons opéré parallèlement avec le cellobiose. La cristallisation mère donne des baguettes plates qui ressemblent en tous points à la maltosazone normale. Ce fait nous a tellement frappés que nous avons recommencé l'expérience plusieurs fois pour nous convaincre qu'il n'y avait pas d'erreur dans les préparations.

Comme dans le cas de la maltosazone, la cellobiosazone nous donne par recristallisation dans l'eau des formes normales: aiguilles en oursins. Tandis que dans l'alcool les formes cristallines sont mal définies et se confondent même avec celles de la maltosazone.

Par recristallisation dans l'eau, la cellobiosazone et la maltosazone présentent donc leurs formes normales quel que soit le nombre de purifications. Il n'en est pas de même dans l'alcool.

En dissolvant dans l'eau un mélange de cellobiosazone et de maltosazone, et en laissant cristalliser lentement, on constate qu'elles conservent chacune leur forme classique, tandis qu'en employant l'alcool comme solvant la cristallisation se rapproche plutôt de celle de la cellobiosazone.

Les points de fusion

Les points de fusion de ces osazones après trois purifications, soit dans l'eau, soit dans l'alcool, et dessiccation dans le vide sur le chlorure de calcium sont:

	au bloc Maquenne	Capillaire
Cellobiosazone	218 à 220° C.	196° C.
Maltosazone	198 à 200° C.	183° C.

Les glucides

Dans les patates

Des patates (300 g.) sont râpées, pressées à travers une toile. Le jus, tamponné à pH 3.7 est agité avec 30 ml. de crème d'alumine et mis à la glacière pendant la nuit. Après centrifugation, le liquide est déféqué à l'acétate de plomb, filtré, et concentré dans des sacs de cellophane à 20° C., dans un courant d'air.

La préparation des osazones fournit: (a) la glucosazone (point de fusion au capillaire, 201° C.; point de fusion mixte, 201.5° C.) et (b) en faible quantité une osazone soluble dans l'eau chaude, que l'on purifie alternativement dans l'alcool et dans l'eau. Suivant les préparations, la forme cristalline est tantôt absente et tantôt se rapproche de celle de la maltosazone; mais le point de fusion varie entre 160 et 170° C. au capillaire. Cette dernière osazone n'est fournie que par l'*Ipomoea*; on ne la trouve pas dans le *Solanum tuberosum*.

Nous avons aussi effectué cette préparation d'osazones avec le liquide de dialyse des macérations faites à la glacière pendant trois jours, soit de poudre de patate, soit de patates râpées.   Les résultats sont identiques.

Dans les produits d'hydrolyse

Aux extraits dialysés et concentrés de jus de patates, on additionne 30 ml. d'amidon à 2.5%, tamponnés à pH 6.7.   Après digestion à 37° C. pendant quatre jours, la coloration avec l'iode est nulle.

La préparation est alors déféquée au moyen d'acétate de plomb et de crème d'alumine, filtrée et concentrée dans des sacs de cellophane.

Les osazones obtenues sont: (a) la glucosazone (point de fusion au capillaire, 202° C.; point de fusion mixte, 201.5° C.) et (b) la maltosazone (point de fusion, 183° C.; point de fusion mixte, 183.5° C.) pour la patate sucrée et la patate ordinaire.   Si nous nous servons, pour l'hydrolyse de l'amidon, des ferments adsorbés par la crème d'alumine à pH 3.7 et élués à pH 7.0, nous obtenons les mêmes osazones.

Commentaires et conclusions

Les extraits dialysés d'*Ipomoea Batatas* et de *Solanum tuberosum* contiennent des amylases; nous désignons celle qui au cours de l'hydrolyse produit du maltose par "maltogène-amylase", et l'autre qui produit du sucrose par "sucrogène-amylase".   Ils contiennent aussi de l'invertase; mais nous n'avons pas réussi à mettre en évidence la maltase, même dans les jus non dialysés.

Dans *Ipomoea Batatas* et *Solanum tuberosum* (à la température de la chambre ou de la glacière), l'action de la sucrogène-amylase est notable, tandis que nous n'avons trouvé aucune trace de l'activité de la maltogène-amylase.   Ces patates contiennent de l'amidon, du sucrose, et des sucres réducteurs: sans doute le mélange glucose-lévulose qui constitue le sucre interverti.   La présence de maltose n'est pas démontrée.

La forme cristalline des osazones conserve son utilité pour l'identification des sucres, pourvu que les conditions de recristallisation soient bien déterminées, par exemple dans l'eau.

L'étude de l'activité amylolytique des jus de patates conduit à toutes sortes de conclusions souvent contradictoires, c'est qu'elle est plus complexe qu'on l'a cru jusqu'à date.   Mettant de côté le rôle de la phosphorylase que nous pouvons inactiver par dialyse (8), nous voyons qu'en plus des facteurs: température, pH, temps et concentration, il faut tenir compte des glucidases présentes; surtout si l'on mesure cette activité par le pouvoir réducteur.

L'hydrolyse enzymatique de l'amidon ne donne pas uniquement des dextrines et du maltose: nous avons un exemple avec les jus d'*Ipomoea Batatas* et de *Solanum tuberosum*.   Nous sommes en mesure d'affirmer que ce phénomène se retrouve dans les fruits tels que: pommes, bananes, et les feuilles de tabac où il y a aussi formation de sucrose.   Ce sucrose, très souvent, se trouve en présence de l'invertase qui l'hydrolyse en proportion plus ou moins grande, suivant les conditions expérimentales.   Il nous semble difficile d'admettre la

conclusion de Nelson et Auchincloss (10) à savoir que le sucrose provient du fructose ou du glucose, à moins que la formation de ces hexoses dépende de la phosphorylase. Mais nous avons contrôlé que notre dialyse rend cette phosphorylase inactive, et Hanes (8) affirme aussi que le jus de patate est dépourvu des enzymes qui président à la conversion phosphoglucose—phosphoglucomutase et isomérase (11).

De plus, le maltose qui est facilement mis en évidence dans l'hydrolyse de l'amidon par les jus dialysés de patates n'a pas pu être identifié dans les patates. L'absence de maltase rend encore plus incertaine la formation d'hexoses.

Toutes ces raisons nous invitent à reconnaître que le sucrose provient de l'amidon au même titre que le maltose et aussi le cellobiose (1, 2) et nous nous croyons justifiables de développer le schéma de Hanes (8), ce qui peut faciliter l'étude de la constitution de l'amidon.
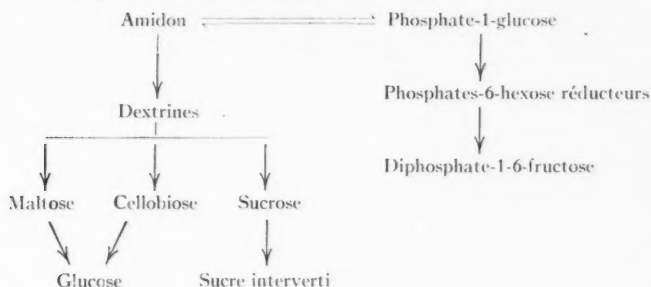
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FACTORS IN THE CULTURE MEDIUM THAT AFFECT THE PHOSPHATASE CONTENT OF YEAST[1]

By E. V. Eastcott[2] and J. J. Rae[3]

Abstract

Yeast grown in an inadequately buffered medium may yield a good crop, but the phosphatase content of the yeast obtained is considerably lower than that of yeast grown in a buffered medium. Wide variations in the magnesium concentration of the growth medium have little effect on the phosphatase content of the yeast obtained. Low phosphate concentrations in tomato juice medium result in a yeast with high phosphatase content.

Since a rapid method was available for the determination of phosphatase in yeast (5), it was decided to investigate the effect of certain factors in the culture medium on the phosphatase content of the yeast.

Materials and Methods

Composition of Culture Media

(*a*) *Tomato juice medium.* The culture medium used was, in the main, that suggested by Clark (1) and consisted of:—

(i) *Sugar and salts:*—Dextrose (5 gm.), ammonium nitrate (0.416 gm.), potassium dihydrogen phosphate (0.208 gm., 0.031 M), magnesium sulphate heptahydrate (0.104 gm., 0.0084 M) and calcium chloride hexahydrate (0.036 gm.);

(ii) *Buffer:*—Citric acid (0.84 gm.), 2 N sodium hydroxide (4 cc.) and N hydrochloric acid (1 cc.);

(iii) *Filtered tomato juice* (15 cc.).

These constituents were mixed and made up to 45 cc. with water. This produces an adequately buffered medium.

(*b*) *Synthetic medium.* In this medium the concentration of the buffer was changed to:—Citric acid (0.504 gm.), 2 N sodium hydroxide (2.4 cc.) and normal hydrochloric acid (0.6 cc.) since less buffer is required than when the acid tomato juice is used, and the filtered tomato juice was replaced by Bios I, inositol (3.4 mg.), Bios IIA, β-alanine (0.017 mg.), *l*-leucine (0.5 mg.), and Bios IIB (15 units). One unit of Bios IIB means that amount, which in excess of all other reagents, in 10 cc. at 25° yields a count of 250 in 24 hr. from an initial count of 1, i.e., 2.5×10^5 cells per cc.

These constituents were mixed and made up to 45 cc. with water. This also produces an adequately buffered medium.

Preparation of Yeast for Analysis

In both cases the culture medium at pH 4.2 was placed in L-shaped rocker tubes, sterilized, and inoculated with 5 cc. of washed yeast suspension (count 10 to 20) (2). Unless otherwise specified the tubes were rocked in a constant temperature bath at 25° for 24 hr. At the end of this time the yeast was centrifuged, washed three times by suspending in water and centrifuging, and dried over calcium chloride in a vacuum desiccator. The yeast was weighed, ground to 40 mesh, and the phosphatase determined by the phenol method (5).

Phosphatase Determinations

(a) TOMATO JUICE MEDIUM

1. *Effect of pH*

Yeast may be grown in a buffered solution or in an inadequately buffered solution. The effect of these conditions on the crop and the phosphatase content of the yeast obtained is shown in Table I. The phosphatase values are the average of duplicate or triplicate determinations.

TABLE I

EFFECT OF BUFFERING OF THE GROWTH MEDIUM ON THE PHOSPHATASE CONTENT OF YEAST

Buffering	Yeast used in inoculation	Time, hr.	pH	Crop, mg.	Phosphatase, mg. phenol liberated*
Adequate	H.P.C.	0	4.20	—	4.25
	H.P.C.	16	4.20	90	3.98
	H.P.C.	23.5	3.83	383	4.28
Inadequate	H.P.C.	0	4.26	—	4.03
	H.P.C.	16	4.05	75	3.11
	H.P.C.	24	2.57	340	0.90
Adequate	L.P.C.	0	4.33	—	0.59
	L.P.C.	13	4.32	21	2.96
	L.P.C.	26	4.09	260	3.46
Inadequate	L.P.C.	0	4.30	—	0.59
	L.P.C.	13	4.21	32	2.41
	L.P.C.	26	2.44	390	0.80

The phosphatase values are the number of milligrams of phenol liberated from a mixture of 10 mg. of yeast and 10 cc. of 0.01 M disodium phenyl phosphate at pH 4.1 after 20 min. at 37° C. They have been corrected for comparative purposes by means of a standard yeast, which was analysed in each series.

The yeast obtained in a buffered medium was found to have a high phosphatase content (H.P.C. Yeast); in an inadequately buffered medium, a low phosphatase content (L.P.C. Yeast). When a culture medium was inoculated with H.P.C. yeast and buffered there was little or no decrease in the phosphatase content of the crop; if it was inadequately buffered there was a marked decrease in phosphatase content. When L.P.C. yeast was used in a

buffered medium there was an increase in phosphatase; in an inadequately buffered medium there was an increase followed by a decrease. When growth was continued beyond the usual 24 hr. the phosphatase content dropped, and this decrease was more marked in the inadequately buffered media. It was found on investigation that the supernatants of yeasts showing a decrease in phosphatase content did not contain an appreciable proportion of phosphatase. On the basis of these results it was decided to use H.P.C. yeast in a buffered medium in subsequent experiments.

2. *Effect of Magnesium and Phosphate*

The results showing the effects of magnesium and phosphate on the tomato juice medium are given in Table II.

TABLE II

EFFECT OF MAGNESIUM AND PHOSPHATE ON THE PHOSPHATASE CONTENT OF YEAST

Magnesium			Phosphate		
Millimoles Mg added	Crop, mg.	Phosphatase, mg. phenol liberated	Millimoles P added	Crop, mg.	Phosphatase, mg. phenol liberated
8	385	3.79	31	409	4.27
4	301	3.62	8	422	4.38
2	348	3.60	4	406	4.53
1	354	3.34	0	236	7.94
0	288	2.59	—	—	—

Although other workers (4) have shown that magnesium activates phosphatase, it was found that the addition of $0.004\ M$ magnesium did not affect the determination of phosphatase in yeast by the phenol method. The sugar and salts reagent contains 8 millimoles of magnesium per litre. When this concentration was reduced by stages to 1 millimole there was no effect on the crop or phosphatase content of the yeast. With no *added* magnesium both crop and phosphatase content were slightly lowered. Filtered tomato juice contains 0.1 mg. of magnesium per cc. This represents a concentration of 1.2 millimoles in the medium.

The sugar and salts reagent contains 31 millimoles of phosphate per litre. When this concentration was reduced by stages to 4 millimoles there was no effect on the crop or the phosphatase content of the yeast obtained. With no *added* phosphate the crop was decreased to about one-half, but the phosphatase content was almost doubled. Filtered tomato juice contains 0.1 mg. phosphate per cc. as determined by King's method (3). This represents a concentration of about 1 millimole in the medium.

In an attempt to investigate further this interesting result a set of experiments was performed in which the phosphate added was reduced gradually from about 4 millimoles to zero. In order to reduce still further the inorganic

phosphate it was precipitated from the tomato juice at pH 10 with magnesia mixture. The phosphate was then replaced by equivalent amounts of potassium dihydrogen phosphate and sodium β-glycerophosphate.

TABLE III

EFFECT OF LOW-PHOSPHATE MEDIA ON THE PHOSPHATASE CONTENT OF YEAST

Tube No.	Milligrams of P added		Crop, mg.	Phosphatase, mg. phenol liberated
	In tomato juice	As KH₂PO₄		
1	1.8	36.6	290	3.70
2	1.8	4.6	277	3.68
3	1.8	2.3	254	3.82
4	1.8	1.2	263	4.88
5	1.8	0.6	244	6.03
6	1.8	0	200	6.57
7	0.86	0	44	6.70
8	0.86	0.73	174	7.10

These results confirm the fact that if the phosphorus is lowered, the phospatase content increases. It is impractical however to lower the phosphorus content much below 0.86 because of the small crop obtained. In Tubes 5 and 6 it was observed that 40% of the total phosphorus remained in the yeast centrifugate. The addition of potassium dihydrogen phosphate or of sodium glycerophosphate (data not shown in the table) to the culture medium in amounts sufficient to restore the original concentration of phosphorus in the tomato juice resulted in an increased crop with a similar high phosphatase content.

(b) SYNTHETIC MEDIUM EXPERIMENTS

High phosphatase content yeast grown in a buffered synthetic medium at 25° C. for 24 hr. yields a yeast whose phosphatase content is 3.06, a value slightly lower than that obtained with tomato juice medium. The effect of magnesium and phosphate on the phosphatase content of yeast grown in a synthetic medium is shown in Table IV.

TABLE IV

EFFECT OF MAGNESIUM AND PHOSPHATE ON THE PHOSPHATASE CONTENT OF YEAST GROWN IN A SYNTHETIC MEDIUM

Magnesium			Phosphate		
Millimoles Mg added	Crop, mg.	Phosphatase, mg. phenol liberated	Millimoles P added	Crop, mg.	Phosphatase, mg. phenol liberated
8	161	3.14	31	135	2.34
1	160	3.07	12	130	1.81
0.5	153	3.35	8	102	2.64
0	No growth	—	4	65	2.47
			0	No growth	—

When the magnesium concentration of the sugar and salts reagent was reduced by stages to 0.5 millimoles there was no effect on the crop or its phosphatase content.   With no added magnesium there was no crop.   When the phosphate concentration was reduced to 4 millimoles the crop was reduced to one-half while the phosphatase content remained about the same.   With no added phosphate there was no crop.   The Bios IIB used contained no phosphorus.

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THE SYSTEM ETHANOL-METHANOL AT 40° C.[1]

By A. C. Morris[2], L. T. Munn[2], and G. Anderson[2]

Abstract

The system ethanol–methanol was studied at 40° C. using the original and also a modified form of the apparatus of Ferguson and Funnell. The total pressure curve is linear and the partial pressure curves agree with Raoult's law within the experimental error.

Introduction

The system ethanol–methanol has been studied by Schmidt (9) at 40° C., and his total pressure curve indicates that there is a slight positive deviation from ideality. Unfortunately his value for the vapour pressure of pure methanol is well below that of other investigators, and because of this fact the extent of the deviation from Raoult's law is still in doubt. A study of this system was undertaken by the authors in order to clear up this point, and also in order to test an improved form of the apparatus of Ferguson and Funnell (3).

Materials

Anhydrous alcohols of reagent quality were dried either by distillation over sodium or by the Lund–Bjerrum method (7).

Experimental Method

Morris used the earlier type of apparatus (2) in which the vapour was circulated over the liquid phase until equilibrium was attained, and a known part of the vapour was then condensed and weighed. The vapour was analysed by means of vapour density measurements, the proper constants being determined experimentally. The composition of the liquid phase was obtained by difference from the amounts of the alcohols initially taken and the amounts that were recovered in the vapour phase.

The apparatus was later modified in order to eliminate the use of mercury traps and to avoid possible errors that might arise from the condensation of mercury in the vapour-phase part of the apparatus. The new form is shown in Fig. 1. Like the older type it was constructed of Pyrex glass. Known weights of the alcohols were placed in the sample tube A through the straight tube which was then sealed. The sample tube was connected to the apparatus by means of the ground glass joint B. The sample was freed of dissolved air by freezing it in liquid air, evacuating, closing the stopcock on the sample tube, and allowing the tube to warm up to room temperature, and then repeating the whole operation several times. It was distilled into the tube C by cooling the latter in liquid air and opening the stopcock, the line to the vacuum pump being closed after the final evacuation. When the distillation
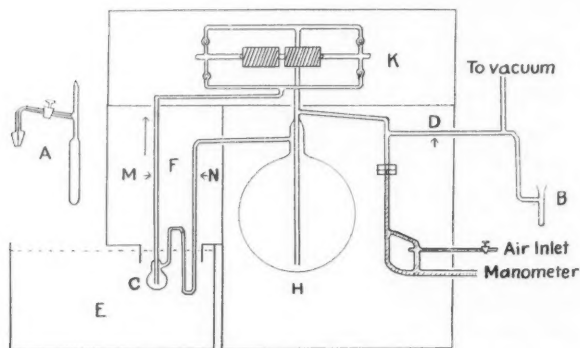
FIG. 1.   *The modified form of the Ferguson–Funnell apparatus.*

was complete, the system was sealed off at the point D.   The bath E was then filled with water and maintained at 40° C. ± 0.01° C.   The air-baths F, H, and K were kept at 45, 44, and 50° C., respectively, and the temperature of H was maintained within ± 0.1° C.   The temperatures were read on mercury thermometers that had been compared with a recently calibrated platinum resistance thermometer which was accurate to better than 0.02° C. Bath F was so designed that glass seals could easily be made at the points M and N at the conclusion of the experiment without undue thermal disturbance of the vapour phase.   Bath K contained the Funnell–Hoover gas pump (4).   Copper sheeting extended from the air-bath F well below the water level in E.   The vapour was circulated over the liquid phase until the pressure became constant, usually a matter of several hours.   The bulb C was then sealed off.   It was dried externally, weighed, opened, dried internally using a slow current of dry air, and then reweighed.   The weight of the liquid phase was obtained from the weight difference by correcting it for air buoyancy and for the vapour that was present.   The latter correction amounted to approximately 0.5% of the vapour, since the large bulb had a volume of five litres.

The new apparatus and procedure differs from the original in that the sample is now distilled directly into the vapour pressure bulb C; the weight of the liquid phase is determined experimentally; no vapour is condensed; and the mercury traps are eliminated.   The composition of the liquid phase is obtained from a total pressure curve, and the composition of the vapour phase is found by difference between the original amounts of the alcohols taken and the amounts recovered in the liquid phase.   In the former procedure the recovery was obtained from the vapour phase.   The mercury-in-glass manometer was sealed to, and was a part of, the all-glass apparatus in both cases.

The apparatus was also used by Munn and Anderson for total pressure measurements but the gas space was reduced by the elimination of the large bulb and the gas circulating pump.   A small vapour phase was especially desirable in the case of the solutions.

Total and Partial Pressures

The total and partial pressure results are given in Table I and are shown in Fig. 2. Munn's values for the vapour pressures of the pure alcohols were used in drawing the total pressure curve. Had the value of Morris for methanol been used, the partial pressures of the latter would have approached more closely the Raoult law line. It should be noted that the total pressures of Morris are independent measurements obtained at the same time that the compositions of the phases were determined.

TABLE I

THE TOTAL AND PARTIAL PRESSURES FOR THE SYSTEM ETHANOL–METHANOL AT 40° C.

Mole per cent methanol		Total pressure, P	Ethanol $\dfrac{n_1}{n_1 + n_2} \cdot P$	Methanol $\dfrac{n_2}{n_1 + n_2} \cdot P$	Observer
Liquid	Vapour				
0	0	135.2	135.2	0	Munn and Anderson
0	0	135.4	135.4	0	Morris
0	0	134.8	134.8	0	Schmidt
14.4	25.2	152.8	114.3	38.5	Morris
26.3	41.0	166.0	97.9	68.1	Morris
31.5		174.6			Munn and Anderson
47.0	64.4	195.0	69.4	125.6	Munn and Anderson
50.2		198.8			Munn and Anderson
65.2		217.6			Munn and Anderson
73.2	84.6	228.5	35.1	193.4	Munn and Anderson
82.7	89.4	238.5	25.4	213.1	Morris
86.0		245.3			Munn and Anderson
89.7	93.3	249.0	16.7	232.3	Morris
100	100	262.6		262.6	Munn and Anderson
100	100	261.5		261.5	Morris
100	100	257.4		257.4	Schmidt

The vapour pressures obtained for ethanol are in agreement with the I.C.T. value of 135.3 mm. (6, p. 217). There is no such agreement between the results of the various investigators in the case of methanol. Schmidt's value is certainly too low. Other values are: (i) Young (11) 260.5; (ii) Ferguson and Funnell (3) 261.7; (iii) Wrewsky (10) 261.9; and (iv) Bredig and Bayer (1) 262.8 mm. Values (ii) and (iii) were extrapolated from 39.90° and value (iv) from 39.76°. The discrepancies are much greater than would be expected, since the greatest care was exercised by these investigators. Without doubt, they are due in part to the presence of water in the alcohol but also in part to other impurities. Munn dried two samples of alcohol by the same method and the densities of the final products were D_4^{25}, 0.7866 and 0.7865 respectively. Gibson's value (5) is 0.78655. The vapour pressures were 262.6 and 265.3 mm. The latter value was checked by the value
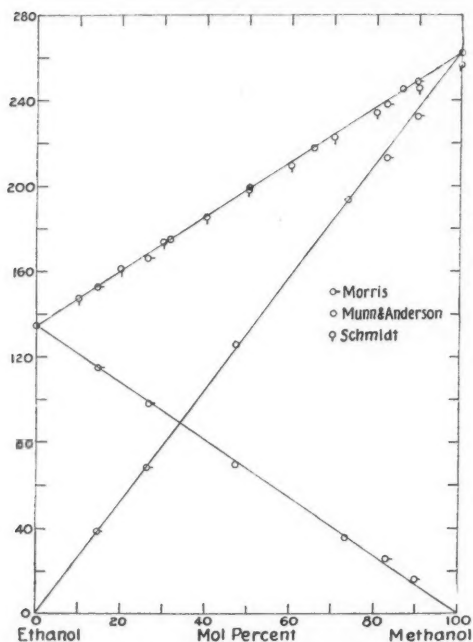
FIG. 2.   The partial and total pressures for the system ethanol–methanol at 40° C.

265.6 obtained for a sample of synthetic alcohol that was said to be very pure but not sold as a reagent chemical.   Evidently the density is not a sufficient criterion of purity, although in general use for this purpose.   The usual chemical tests also do not seem to be satisfactory since the various workers tested their alcohols.

Since the partial pressure curves indicate that the system closely approaches an ideal one, the deviation from ideality of one alcohol must be matched by the deviation on the part of the other alcohol.   If we assume an equation of state for the vapour of the type $P (V-\beta) = RT$, the values of β may be calculated by means of the equation of Scatchard and Raymond (8).   These, in turn, yield formula-weight deviations from the theoretical values, of 0.7 and 1.1% for ethanol and methanol respectively.   This gives an approximate idea of the contribution of the vapour to the deviations.   The experimental vapour densities as determined in this laboratory agree very well with those so calculated for ethanol and acetone, but those for methanol are invariably much higher.   The writers hesitate to accept as final, formula-weight values for methanol as low as 32.4 at 50° C., and these investigations are being continued. The theoretical values were used in calculating the mole percentages given in the table.

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THE SYNERESIS OF SILICA GELS CONTAINING ADDITION AGENTS[1]

By L. A. Munro[2] and G. E. Monteith[3]

Abstract

The effect of addition agents on syneresis of silica gel is reviewed. Various methods of measurement of syneresis have been investigated and a convenient method that gave duplicate results agreeing within 1.2% is described. The effect of a series of alcohols—methyl, ethyl, glycol, glycerol, and erythritol—on syneresis has been studied. The results indicate that the total volume of syneretic liquid at equilibrium was the same as that of the control (within the experimental error) and that no adsorption had taken place in the gel itself. The data on the adsorption of the alcohols was determined by analysis of the syneretic liquid for its alcohol concentration by an oxidation method using chromic acid.

Introduction

The effects of various neutral organic addition agents on the time of set of silica gels have been shown to be widely divergent for alkaline gels (12, 13). In a study using a series of monohydric and polyhydric alcohols it was noted that when the system was acidic all the alcohols acted as retarders of gelation. In the alkaline range the monohydric alcohols and glycol were accelerators. At pH 7 all had a uniform and retarding effect; this eliminated therefore the dielectric constant of the addition agent as the principal factor in determining gelation. If the specific effects observed on the alkaline gels are to be explained by differences in sorption of the addition agents or in the solvation of the micelles during gelation, this should be indicated by the composition of the syneretic fluid. The work described below was undertaken to investigate this, using as addition agents the series of alcohols studied in the experiments on the time of set.

The work on syneresis and the theories concerning it advanced up to 1929 have been reviewed by Kuhn* (8). Evidently considering the gelation phenomenon to cover the life history of a gel until an equilibrium is finally reached, Kuhn depicts syneresis as a continuation of the disintegration process following setting by structural changes and desolvation. A perusal of Kuhn's paper will show the great differences in behaviour of different types of gels; for example, rubber, silicic acid, and viscose give increasing amounts of syneresis with increasing concentration of gel, whereas the opposite behaviour is reported for starch, agar, cellulose acetate, and dyestuffs.

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*One important part of Kuhn's paper, viz., a discussion of Liepatoff's work is in error owing to certain mistakes of translation and interpretation. See Liepatoff (10).

Syneresis of silica gels has been reported by several investigators, but the results and conclusions are not always in agreement. This may be due partly to different methods of measurement, and partly to the neglect of such factors as pH, temperature control, and total surface per cubic centimetre. Fracture of the gel or mechanical disturbance causes differences in the rate and amount of syneresis.

The effect of addition agents on syneresis has been recorded in only a few cases. Rossi and Marescotti (16, 17), Rossi and Scandellari (18), and Jirgensons (7) have noted the effect of electrolytes on agar, starch, and albumin gels.

The effect of electrolytes on syneresis has been noted for various inorganic gels by Prakash and Dhar (14).

Bonnell (2) studied the effect of neutral salts on silica gel syneresis. The work was done at a pH of 6.5 and at 20° C. The added salts increase the rate and final volume of syneresis up to a maximum which appears to be between 0 and 1.353% in the case of potassium chloride at a silica concentration of 8.4%. It was found that potassium salts are more potent than sodium salts and the sulphates better than the chlorides.

Hardy (4), using azomethine, observed that, in an ether gel, syneresis occurs in a few minutes, in absolute alcohol in a day, and in aldehyde in a few hours. Slight additions of water retard syneresis from an hour to a day. Alcohol added to a gel containing salt gives increasing syneresis in a short period of time, but the end-point is the same as for gels containing electrolyte alone. The effect of organic addition agents on syneresis of silica gel has apparently escaped attention.

Experimental

A comparison of three methods of measurement was first undertaken. In Method 1 the apparatus described by Ferguson and Applebey was used (3). This consisted of a cylinder of 50 cc. capacity having a graduated neck made from an old burette. The bottom of the cylinder was connected to a reservoir bulb. It is labelled No. 1 in Fig. 1. In this method a standard volume of
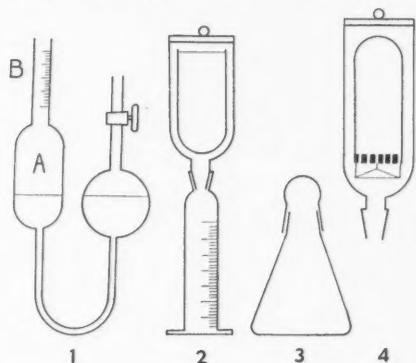

FIG. 1. *Types of apparatus used in the different studies.*

gel is formed in Tube *A* on a platform of carbon tetrachloride. The synereticum is measured by raising the level of the carbon tetrachloride and reading the volume of liquid displaced into the graduated tube, *B*.

The second method made use of a simplification of the apparatus described by Bonnell (1) (No. 2, Fig. 1). The gel was formed in a vaselined extraction thimble and as soon as it had set it was transferred to the experimental one, saturated with syneretic liquid from a similar gel.

In the third method, uniform Erlenmeyers having the same internal surface were coated with wax. When the gel had synerized, the flask was inverted and the volume of liquid measured at different times.

A comparison of the results for a 1.65% silicate gel (made from 25 cc. of silicate, containing 1.65% silica, and 25 cc. of acetic acid) at pH 11.0 and 20° C. is shown in Fig. 2. These curves represent the mean of several readings.
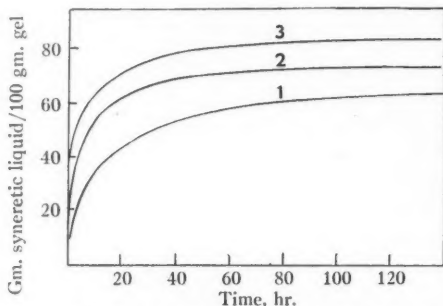

Fig. 2. *Syneresis curves obtained by different methods. Curve 1—Ferguson and Applebey method. Curve 2—Bonnell wetted cone or thimble method. Curve 3—Waxed Erlenmeyer method.*

Factors Contributing to Experimental Differences

Holmes *et al.* (6) found that the amount of syneresis per cubic centimetre varied with the surface. Ferguson and Applebey (3) found that the same volume of gel in containers of different shapes, viz., a sphere, cylinder, cone, and cuboid, gave a maximum difference of 6% in the volume of liquid, although the surface area varied as much as 35%. It is indeed probable that the volumes of syneretic liquid are within the experimental error. The differences *herein* noted must therefore be due to other factors inherent in the three methods. The gel used in these experiments was more dilute and differences in height of the gel and thus in compression may be of more importance.

Kuhn states, "Mechanical influences affect very strongly the spontaneous exudation of liquid from gels. If a gel which shows no noticeable syneresis is subjected to the slightest pressure it loses liquid; for example, soaps (aqueous alcohol and glycerine)." Meyer (11) has described similar observations with starch. A 5% starch gel which was stored away quietly for two months

exuded 1 cc. of liquid; a similar gel, however, after 48 hr. during which it was given an occasional feeble shake had exuded liquid, and after seven days 6 cc. of liquid was freed.   A 6% gel had exuded no liquid after eight days of quiet standing; when a rod, held upright in the gel, was moved slightly, however, the gel was torn, and it collapsed.   The 'fluid' produced by this mechanical disintegration is not due to syneresis, but, partly at least, to thixotropic unsettling.

Further methods for the measurement of syneresis were tried and every precaution was taken to achieve similarity of conditions.   First, the gel itself was allowed to synerize in a waxed seamless Whatman extraction thimble, 80 mm. high and 22 mm. in diameter, with a hemispherical bottom.   The container was saturated by boiling it in wax for one hour, and then allowing it to cool.   This expelled nearly all the trapped air.   The cone was then dipped twice in warmed wax and a uniform coating was thus formed.   A hole 2.5 mm. in diameter was punched in the centre of the bottom of the thimble, and after the final dipping this was reamed out to a diameter of 2.0 mm.

When the apparatus was about to be used, a circular piece of drafting-tape was sealed over the hole in the cone or thimble by means of a thin coating of wax.   The sol was then pipetted into the thimble and allowed to set.   The piece of drafting tape was then removed, the thimble placed in the apparatus and the syneretic liquid collected in the graduated cylinder (No. 2, Fig. 1).

Results with this apparatus were not very satisfactory, particularly with acid gels.   Data for alkaline gels from '3.35% silica' silicate obtained by J. A. Pearce in this laboratory at pH 10.5 are given in Table I.   Gelating mixture (50 cc.) was prepared as described previously and 10 cc. of the mixture pipetted into the waxed thimble.   The time was measured from the instant of mixing.

A further modification illustrated as No. 4, Fig. 1, was then tried.   Wax discs, 0.5 to 2.0 mm. thick, were cast to fit the extraction thimble.   These

TABLE I

DATA OBTAINED WITH '3.35% SILICA' GELS, AT 20° C., pH 10.5

Addition agent	Hours				
	23	44	69	118	163
	Volume of syneretic liquid, cc.				
Control	8.3	8.5	8.5	8.5	8.6
	8.3	8.3	8.5	8.7	8.7
Glycol, 3 cc.	8.5	8.8	8.9	9.0	9.0
	8.5	8.8	8.8	8.8	8.9
Glycerol, 3 cc.	8.8	9.0	9.0	9.0	9.0
	8.4	8.7	8.7	8.8	8.8
Erythritol*, 4 gm.	8.8	9.0	9.0	9.0	9.0
Mannitol, 4 gm.	8.0	8.1	8.3	8.3	8.3
	7.9	8.1	8.3	8.3	8.3

In the case of the second sample, some silica came through and precipitated on the bottom of the graduate.

discs were pierced uniformly with 15 holes 1 mm. in diameter.   When the gel mixture had been pipetted into the thimble the disc was inserted in the top and sealed on by means of a hot knife-blade.   As soon as the gel had set, the container was inverted and the hole in the bottom opened to act as an air vent.   The inverted thimble was supported on three glass points.   This apparatus gave the most consistent results.

Syneresis was virtually complete after 20 days, whereas with the other apparatus syneresis was still proceeding after 90 days.   The total volume of syneresis was also greater with this method, and the final volumes checked fairly well.

However, near the end of the syneresis period the holes have a tendency to plug up, possibly owing to secondary gelation.   This factor may cause differences in the final volumes of duplicate samples.   The other possibility is that the syneretic liquid cannot escape with the same ease from all the thimbles.

Preparation of the Sol

In these experiments, Baker's Crystalline silicate and freshly distilled water were used.   The silicate had a specific gravity of 1.050 at 20° C., and it contained 2.5% of SiO_2.   The acid solution was prepared by adding 50.0 cc. of glacial acetic acid to distilled water and making up to 500 cc., giving a normality of 1.63.

To obtain consistent results the method of mixing is important.   The silicate solution is always added to the acid solution.   The required amount of acid is measured from a burette and made up to 25 cc. with water or addition agent plus water.   Silicate solution (25 cc.) is then added with stirring for five seconds.   When crystalline alcohols were added, the weighed amount was dissolved in the acid and the volume of the mixture made up to 50 cc.

Ferguson and Applebey (3) have shown that temperature has a marked effect on syneresis, a 10° rise increasing the amount about 1.8 times.   Bonnell verified this, using hydrochloric acid and acetic acid as coagulants.

Since there is, owing to the heat of mixing, a definite rise in temperature that might influence the syneresis, the separate silicate and acid solutions were kept at a temperature below that of the bath (25° C.) containing the apparatus.   The correct premixing temperature for gels of pH 10.4 was found to be 22.3° C.; for gels of pH 9.4, 21.7° C.; and for gels of pH 7.4, 21.6° C.

To check the similarity of these gels with those previously studied, times of set were determined, as previously described, with the residual mixture after removal of the pipetted sample for the syneresis experiment.   The gel concentration used was much lower than concentrations used by other experimenters in this field.   At this dilution, gels never set in less than five minutes, which is ample time for the necessary manipulation.

The tubes were placed in an air thermostat regulated to 25° C., within 0.05° C., by means of a sensitive mercury-toluene thermoregulator operating a 40 watt lamp. A small fan revolved behind the lamp. The motor and shaft of the fan were mounted on a nearby table so as to avoid any vibration of the fragile gel.

Relation of pH and Syneresis

The change in pH of a gel on setting has already been noted (12). Several investigators have recorded the influence of pH on syneretic volume for silica gels. Kuhn (8) states that neutral gels showed weak syneresis, whereas basic gels which set rapidly, synerize quickly. Ferguson and Applebey showed that whereas acid gels (without addition agents) synerize more slowly, the final quantity of synereticum is greater than with alkaline gels.

However, the pH at which syneresis is a minimum depends on the concentration of the gel. Bonnell, using concentrated gels containing 6.5 to 8.8% silica, showed that the volume of liquid synerized passes through a minimum at about pH 8.5, increasing steeply beyond 10. Ferguson and Applebey, using a 3.87% silica gel and pH 5, 7, 9, and 10 found the smallest volume at pH 10. They observed that at pH 4 to 5 more concentrated gels gave greater syneresis. It has been shown (12) that the pH at which the time of set is a minimum depends on the concentration of the gel, varying from pH 7.5 to pH 10 as the percentage of silica was increased. The discrepancies between Bonnell's and Ferguson and Applebey's results may be due to the concentration factor.

In the present studies both the glass electrode and indicators were used. It was found advisable to have a standard solution to check the glass electrode, as in the strongly alkaline range it gives somewhat erroneous results. Check values for the two methods varied less than 0.1 pH unit.

It is noted that the gel becomes much more alkaline during the period between the time of mixing and that of the setting of the gel. A number of gelating mixtures of pH 9.0 gave, on setting, gels of pH 9.6 to 9.7. The liquid exuded by syneresis was withdrawn every 12 hr. and its pH recorded. The synerized liquid was of a constant pH, throughout the whole period of syneresis, as recorded by the Coleman glass electrode.

Analysis of Syneretic Liquid

The syneretic liquid was analysed to determine whether the gel adsorbed any of the alcohol while setting, and to study the further processes taking place during syneresis.

The oxidation method essentially similar to that given for glycerol in Lawrie's book "Glycerol and the glycols" (9, p. 238) was employed. This is based on Hehner's method (5). A standard concentrated solution of chromic acid (79.86 gm. of potassium dichromate and 200 cc. of concentrated sulphuric acid per litre) was used to oxidize the different alcohols, the excess dichromate being titrated with ferrous ammonium sulphate. Twenty-five cc. of ferrous

ammonium sulphate was equivalent to 10 cc. of chromic acid solution.   From 50 to 100% excess chromic acid was measured out accurately in duplicate into 250 cc. Erlenmeyer flasks (approximately 40.0 cc. for erythritol and 20 cc. for methyl alcohol).   Five cc. of the syneretic liquid was then pipetted into the flask, which was quickly placed under a reflux condenser to avoid loss of the lower alcohols by evaporation.   A volume of concentrated sulphuric acid equal to the chromic acid solution was then added slowly down the reflux condenser.   The heat generated starts the reaction vigorously.   The flask was finally heated over a boiling water-bath for $\frac{1}{2}$ hour to insure completion of the reaction.   The flask was then cooled and the contents titrated with ferrous ammonium sulphate, 0.1% potassium ferricyanide solution being used as an outside indicator.
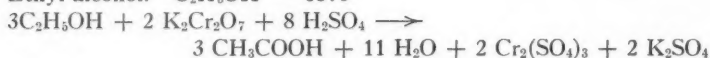
Richardson and Jaffé (15) found that by increasing the amount of sulphuric acid added, the time of boiling (two hours at least was recommended by Hehner and Lawrie) could be greatly reduced.   The writers have found one-half hour to be ample.   As can be seen from the equations, large quantities of sulphuric acid enter into the reaction.

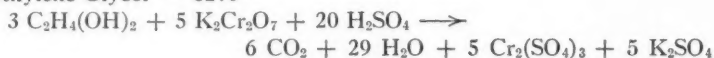*Reactions with $K_2Cr_2O_7$.   Potassium dichromate. Mol. wt. 294.22*

Methyl alcohol. $CH_3OH - 32.0$

$$3\ CH_3OH + 2\ K_2Cr_2O_7 + 4\ H_2SO_4 \longrightarrow$$
$$CO_2 + 6\ H_2O + Cr_2(SO_4)_3 + K_2SO_4$$
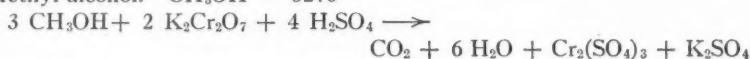
Ethyl alcohol. $C_2H_5OH - 46.0$
$$3C_2H_5OH + 2\ K_2Cr_2O_7 + 8\ H_2SO_4 \longrightarrow$$
$$3\ CH_3COOH + 11\ H_2O + 2\ Cr_2(SO_4)_3 + 2\ K_2SO_4$$

Ethylene Glycol − 62.0
$$3\ C_2H_4(OH)_2 + 5\ K_2Cr_2O_7 + 20\ H_2SO_4 \longrightarrow$$
$$6\ CO_2 + 29\ H_2O + 5\ Cr_2(SO_4)_3 + 5\ K_2SO_4$$

Glycerol − 92.08
$$3\ C_3H_5(OH)_3 + 7\ K_2Cr_2O_7 + 28\ H_2SO_4 \longrightarrow$$
$$9\ CO_2 + 40\ H_2O + 7\ Cr_2(SO_4)_3 + 7\ K_2SO_4$$

Erythitol − 122.08
$$C_4H_6(OH)_4 + 3\ K_2Cr_2O_7 + 12\ H_2SO_4 \longrightarrow$$
$$4\ CO_2 + 3\ Cr_2(SO_4)_3 + 3\ K_2SO_4 + 17\ H_2O$$
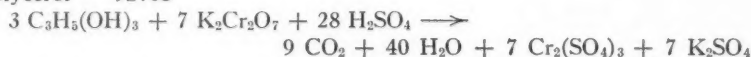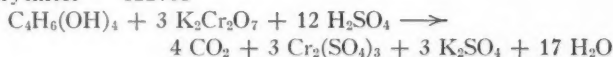
It is noted that all the above alcohols are oxidized completely to carbon dioxide, except ethyl alcohol, which is oxidized to acetic acid.

Tabulation of Results

The results are given in Table II.   In Column 4 are given the average volumes of syneretic liquid from the duplicate experiments, the extent of deviation between Tubes I and II being shown.   With this type of apparatus the maximum deviation between duplicates was ± 0.3 cc. or 1.2%, while

the variation from the value for the control was ± 0.5 cc. or 2.9%. The slight variations observed in pH apparently did not affect the results. In a second control gel of pH 8.0 the syneresis was approximately 5 cc. per 100 gm. less than the control at pH 9.7.

In the last two columns are given the amount of addition agent in the syneretic liquid and in the original gelation mixture. Very good agreement was obtained between duplicate titrations and between duplicate experiments (Tubes I and II) except in the case of methyl alcohol and Tube II of erythritol.

TABLE II

DATA OBTAINED WITH '2.5% SILICA' GEL AT 25° C, PH 9.7, USING IMPROVED APPARATUS (NO.4)

	pH		Volume of syneretic liquid from 25 cc. of gel	Volume as cc./100	Addition agent	
	At set	Of syneretic liquid			Gm./100 cc. syneretic liquid	Gm./100 cc. original mixture
Control	9.7	9.7	17.4 ± 0.0	69.6	0	0
Methyl alcohol	9.7	9.8	17.3 ± 0.3	69.2	1.57	1.60
Ethyl alcohol	9.7	9.85	17.9 ± 0.1	71.6	2.30	2.30
Glycol	9.7	9.7	16.8 ± 0.2	67.2	3.12	3.12
Glycerol	9.7	9.7	17.4 ± 0.0	69.6	4.52	4.52
Erythritol	9.7	9.7	17.9 ± 0.1	71.6	6.10⎫ 5.93⎭	6.10
Control	7.9	8.0	16.1 ± 0.1	64.4	—	—

Two typical examples that show the accuracy of the method are given below.

Example Titrations

Ethyl alcohol, 2.302 gm. per 100 cc.   Sample of synereticum, 5 cc.

From Tube I *(a)* 15.00 cc. of dichromate required *(a)* 22.20 cc., *(b)* 22.30 cc. ferrous ammonium sulphate = 0.1150 gm., 0.1145 gm. ethyl alcohol.

From Tube II *(c)* 15.00 cc. dichromate required 22.25 cc., 22.10 cc. ferrous ammonium sulphate = 0.1147 gm., 0.1152 gm. ethyl alcohol or 2.297 gm. per 100 cc.

Glycol, 3.120 gm. per 100 cc.

5 cc. from Tube I, using 25.00 cc. dichromate, required 23.90, 23.90 cc. Tube II, using 25.00 cc. dichromate required 23.90, 23.80 cc. = 0.1560 gm. of glycol or 3.120 gm. per 100 cc.

The ethylene glycol used was 97.1% pure and the glycerol was 94.2% pure. This degree of purity is quite normal as it is difficult to remove the last traces of water from these alcohols.

It will be seen from the analysis of the syneretic liquid given above that no selective adsorption of the alcohols has taken place.   The syneretic liquids all contained silica in undetermined amounts.

It appears that the specific effect of these addition agents on the time of set and the gelation process is not indicated by the composition of the syneretic fluid.

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THE HYDROLYSIS OF ALIPHATIC NITRILES IN CONCENTRATED HYDROCHLORIC ACID SOLUTIONS[1]

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Abstract

Studies of nitrile hydrolysis in concentrated acid media have been extended to the hydrolysis of hydrocyanic acid, acetonitrile, and cyanoacetic acid in hydrochloric acid solutions of concentration 1 to 8.5 N. It appears that the hydrolysis of aliphatic nitriles in concentrated sulphuric and hydrochloric acids provides systems of consecutive, irreversible unimolecular reactions over the complete range $k_{nitrile} \ll k_{amide}$ (more dilute acid) to $k_{nitrile} \gg k_{amide}$ (highly concentrated acid), for a sufficient range of acid concentrations. The limit, $k_{nitrile} \gg k_{amide}$, is not attained in hydrobromic acid solutions. The nitriles studied here exhibit a large decrease in activation energy with increase of acid concentration, similar to that observed earlier for propionitrile. The magnitude of the variation of the Arrhenius parameters is specific for each nitrile. For the acetonitrile hydrolysis, the increase in rate with increase of acid concentration largely follows the accompanying activation energy decrease. The relative rates of hydrolysis of different nitriles, at given acid concentration, are not governed by differences in activation energy. The variation of activation energy is briefly discussed in relation to certain other factors.

Introduction

It was found in a previous investigation (16) that the activation energy for the hydrolysis of propionitrile in concentrated hydrochloric acid solutions decreases by 6.7 Cal. with increase of acid concentration from 1 to 10 N. The very rapid increase in rate observed in this concentration range is largely accounted for by the decrease in activation energy. This behaviour was found to be general for the reaction in concentrated mineral acid solutions (13).

The study of propionitrile has also shown that at higher acid concentrations (above 4 N), it cannot be assumed, as other investigators have done (8), that the rate of hydrolysis is independent of the hydrolysis of the intermediate amide. Consequently, the reaction rate should not be measured by determination of ammonia alone, but by analysis for total amide and ammonia. It was found that the reactions, propionitrile $+ \ H_2O \ \xrightarrow{k_1}$ propionamide $+ \ H_2O \ \xrightarrow{k_2}$ propionic acid $+$ ammonia, constitute a system of consecutive, irreversible, unimolecular reactions for which the ratio, $k_1 : k_2$, varies with the catalyst and its concentration. For hydrochloric and sulphuric acids, variation of the rate ratio over the complete range, $k_1 \ll k_2$ (1 M acid) to $k_1 \gg k_2$ (above 11 M acid), was observed.

The study has been extended here to include the hydrolysis of acetonitrile and hydrocyanic and cyanoacetic acids in hydrochloric acid solutions. This has permitted a more general examination of the correlation between increase in rate of hydrolysis with increasing acid concentration and variation of the

Arrhenius parameters.   Since the structure and characteristics of the nitriles employed vary considerably, it was expected that variation of activation energy with acid concentration would be specific.   It was of particular interest to determine whether E varies in the hydrocyanic acid hydrolysis, as the increase of rate with hydrochloric acid concentration for this nitrile has been related by other workers (9) to the square of the mean ion activity of the acid.

Experimental

Acetonitrile was obtained commercially and was purified by distillation to a boiling range of $0.1°$ C.

Hydrocyanic was prepared by a standard method (20), and was further purified by repeated distillation between two traps, the first and last fractions being rejected.   Aqueous solutions were stabilized by addition of a drop of dilute acid (12) and were stored in an ice-box.

Cyanoacetic acid was prepared according to directions in Organic Syntheses (4).   It was purified by recrystallization from water and was dried over phosphorus pentoxide.   Some difficulty was experienced in the purification of this material and the final product, as shown by analysis for ammonia, was 98% pure.   The major impurity is sodium chloride, and, since dilute nitrile solutions were employed, its effect was undoubtedly negligible.

Solutions of nitriles, other than cyanoacetic acid, were prepared by direct weighing.   For cyanoacetic acid, solutions of approximately the desired concentration were standardized by complete hydrolysis and determination of ammonia.   Reaction mixtures were approximately $0.2\ N$ with respect to nitrile.

Other reagents, procedures, and methods of analysis have been previously described (16).

Results

With the exception of cyanoacetic acid, the consecutive reactions involved in the hydrolysis of the nitriles considered here have been investigated by following the reactions through determination of ammonia, and of total amide and ammonia.   The general behaviour is similar to that observed for propionitrile.   The relations between rates of nitrile and amide hydrolysis conform to the treatment previously discussed (16).   Presentation of these experimental data in detail would therefore seem to be superfluous.   The results are summarized in the form of relative rates given in Table I.

Acetonitrile, like propionitrile, provides a system of two irreversible, unimolecular consecutive reactions over the complete range $k_{nitrile} \ll k_{amide}$ (below $4\ N$ acid) to $k_{nitrile} \gg k_{amide}$ (estimated above $11\ N$ acid).   This is seen from Table I, where the variations of the rate ratios with acid concentration

TABLE I

RATE RATIOS OF NITRILE AND AMIDE* HYDROLYSIS AT VARIOUS ACID CONCENTRATIONS

System	Hydrochloric acid concentration, normality					
	1.00	4.00	5.02	6.48	8.47	10.13
Propionitrile: propionamide†	1 : 2000	1 : 300	1 : 90	1 : 10	3 : 1	40 : 1
Acetonitrile: acetamide	1 : 2500	1 : 350	1 : 80‡		2 : 1	
Hydrocyanic acid: formamide	1 : 12000	1 : 500§			1 : 3	

* Reference (15).

† Reference (16).

‡ k_{amide} obtained by interpolation of neighbouring concentrations.

§ $k_{nitrile}$ obtained from Peiker, Ph.D. thesis, McGill University.

are evidently parallel for propionitrile and acetonitrile. Since the rate of acetonitrile hydrolysis increases markedly in the acid range around 8 N, while that of acetamide hydrolysis decreases (6), $k_{acetonitrile}$ will greatly exceed $k_{acetamide}$ at some higher acid concentration, probably above 11 N by analogy with propionitrile.

The behaviour of hydrocyanic acid is slightly different. At an acid concentration of 8.47 N, the rate of hydrolysis of formamide is faster than that of the nitrile, although it has been almost overtaken. Values of k_{HCN} at this acid concentration, as determined by analysis for ammonia, are less than, but approach, the true value. It is evident that up to concentrations of 7 to 8 N the nitrile reaction may be followed by determination of ammonia alone. Since the velocity of hydrolysis of hydrocyanic acid increases so markedly with increase of acid concentration, while that of the amide decreases, such a procedure at acid concentrations above 11 N might result in the evaluation of $k_{formamide}$. As the *trends* in rates are clearly evident, it has not appeared necessary to evaluate precise velocity ratios at acid concentrations other than those employed here.

For cyanoacetic acid, the determination of ammonia alone gives satisfactory velocity constants at acid concentrations of 1 and 4 N. At 8.47 N, however, the familiar, prominent induction period occurs. Evidently, the behaviour of the system, cyanoacetic-acid–malonamic-acid, is similar to that of the other nitriles. Since cyanoacetic is a solid, the analytical technique previously employed for analysis of total amide and ammonia is unsuitable and the reaction velocity has been studied only at acid concentrations of 1 and 4 N. Velocity data for malonamic acid hydrolysis are not available, and it is not possible, therefore, to calculate $k_{nitrile}$ at the higher acid concentrations from analytical data on ammonia production.

It is evident that for a sufficient range of acid concentrations, the consecutive reactions for the nitriles studied in the present investigation have characteristics similar to the unique behaviour observed for propionitrile in hydrochloric and sulphuric acids.

The unimolecular velocity constants for a range of temperatures and acid concentrations, as determined by analysis for total ammonia and amide, are recorded in Table II. The constants are the mean, usually, of five to six

TABLE II

UNIMOLECULAR VELOCITY CONSTANTS FOR THE HYDROLYSIS OF HYDROCYANIC ACID, ACETONITRILE, AND CYANOACETIC ACID AT VARIOUS CONCENTRATIONS OF HYDROCHLORIC ACID

	Concentration of hydrochloric acid, normality	Concentration of nitrile, normality	Temperature, ° C.	$k \times 10^2$ hr.$^{-1}$
Hydrocyanic acid	1.00	0.4365	65.0	0.180*
			80.3	0.869
			99.9	5.64
	8.47	0.2204	0.0	0.855
			19.8	12.6
			29.7	39.3
			34.7	73.8
Acetonitrile	0.509	0.2011	59.6	0.0132
	1.00	0.2012	59.6	0.0292
			83.5	0.428
			93.3	1.16
			99.7	2.28
			111.8	6.65
	2.00	0.2013	59.6	0.0774
	4.00	0.2014	49.6	0.100
			59.6	0.329
			80.6	3.38
			94.1	13.8
			99.5	22.7
	5.02	0.2315	59.6	0.958
			70.1	3.06
			83.1	10.7
			99.7	49.3
	8.47	0.2217	28.4	2.08
			42.7	9.86
			49.6	20.5
			59.6	54.9
Cyanoacetic acid	1.00	0.1851	99.5	1.01
			110.2	2.67
			125.4	10.2
	4.00	0.1855	59.6	0.225
			90.5	5.65
			99.5	13.5
			109.9	36.5

* Value obtained from Peiker, Ph.D. thesis, McGill University.

individual values. The average deviation from the mean was approximately 2 to 3% for cyanoacetic acid and not more than 1 to 2% for the other nitriles. The values at the more dilute concentrations are the more accurate. All concentrations refer to 25° C., and no correction has been applied for variation of collision number with temperature. Errors arising from these approximations will partially cancel and should be negligible for comparative purposes.

The values of A and E calculated from the Arrhenius relations are given in Table III together with values for propionitrile. Values of k/N, expressed in seconds, were employed in the evaluation of A. The error in E is less than 100 to 200 cal. for acetonitrile at 1 and 4 N acid, as determined by comparison of worst and best Arrhenius lines, and less than 300 to 400 cal. for the remaining data. A value of E of 25,300 cal., based on two temperatures only, is recorded for acetonitrile in alcohol–water medium at hydrochloric acid concentration of 0.5 N (5). The hydrolysis of hydrocyanic acid in hydrochloric acid of concentrations up to 7.84 N has been studied over a range of temperatures (7), but neither the rate data nor the precise temperature coefficients are recorded. The statement of Krieble and Peiker (9) that the relative rates of hydrolysis of hydrocyanic acid at different acid concentrations are constant over a temperature range is not in agreement with the present results.

TABLE III

THE ARRHENIUS CONSTANTS FOR THE HYDROLYSIS OF HYDROCYANIC ACID, ACETONITRILE, AND CYANOACETIC ACID AT VARIOUS ACID CONCENTRATIONS

Nitrile	Acid concentration, N	$\log_{10} A$	E, cal.
Hydrocyanic acid	1.00	9.65	24,700
	2.00 ⎫	—	24,300*
	4.00 ⎭		
	8.47	10.65	21,500
Acetonitrile	1.00	10.33	26,500
	4.00	10.30	25,800
	5.02	9.60	24,200
	8.47	9.12	21,100
Cyanoacetic acid	1.00	9.9	26,300
	4.00	9.9	25,500
Propionitrile	1.00	10.04	25,600
	4.00	9.95	24,800
	5.02	9.68	23,900
	6.48	8.92	21,800
	8.47	8.85	20,100
	10.13	8.78	18,900

* *Average value calculated from results of Peiker, Ph.D. thesis, McGill University.*

In general, the magnitude of A found in the present series of studies of nitrile hydrolysis is within a factor of 100 of the so-called "normal" value, on the "slow" side.

Discussion

The nitriles considered in this work have been studied in hydrochloric acid by other investigators (7, 8). They assumed that the velocity of amide hydrolysis is much faster than that of the nitrile over an acid range comparable with that employed here, and they followed the reactions by determination of ammonia. This appears to be satisfactory for hydrocyanic acid since their acid concentration did not exceed $7.84\ N$. However, the results obtained here for acetonitrile, and comparison of their data with those for acetamide (15), show that at a hydrochloric acid concentration of 10 M they have essentially measured the hydrolysis of acetamide. Exact comparison of the present results with their data is difficult because of the inaccuracies of interpolation and because the exact amount of amide hydrolysis occurring during their analysis for ammonia is unknown.

On the basis of the present work and the behaviour of propionitrile (13), it is possible to infer something of the nature of the consecutive reactions involved in the hydrolysis of acetonitrile, hydrocyanic acid, and cyanoacetic acid in mineral acids other than hydrochloric. The reactions in sulphuric and hydrobromic acids are of particular interest since these acids have also been employed by others (8, 9) in studies with these nitriles. In sulphuric acid, the rate relation $k_{nitrile} \ll k_{amide}$ should obtain in the more dilute solutions (about 1 to 4 N). Since the rate of nitrile hydrolysis in sulphuric acid solutions increases more slowly with acid concentration than in hydrochloric acid solutions (8, 16), the rate of hydrolysis of each nitrile should become comparable with that of the corresponding amide at somewhat higher acid concentrations than for hydrochloric acid. Again, since in sulphuric acid solutions of concentration above 20 N the relation $k_{propionitrile} \gg k_{propionamide}$ is found, it might be expected that this rate relation will also be approached for the other nitriles at very high sulphuric acid concentrations. This is supported by the well known fact that amides may be isolated quantitatively from nitriles dissolved in concentrated sulphuric acid.

As with sulphuric acid, the rate of nitrile hydrolysis in hydrobromic acid should become comparable with that of amide hydrolysis at some acid concentration higher than that in hydrochloric acid. However, at the restricted upper limit of hydrobromic acid concentrations ordinarily available (8 N), the relation $k_{nitrile} \gg k_{amide}$ would not be attained.

The relative rates of hydrolysis of different nitriles, at given hydrochloric acid concentration, are not governed even in a general way by the differences in activation energy. This may be seen from Fig. 1, where a linear relation of theoretical slope is obviously not obtained. Deviations from the theoretical relation may be attributed to variations in the A factor, which result from the proximity of the substituent to the seat of reaction and are characteristic of the effect of structural change in most reactions of aliphatic compounds (18). Simultaneous variations of A and E make analysis of the relation between rate
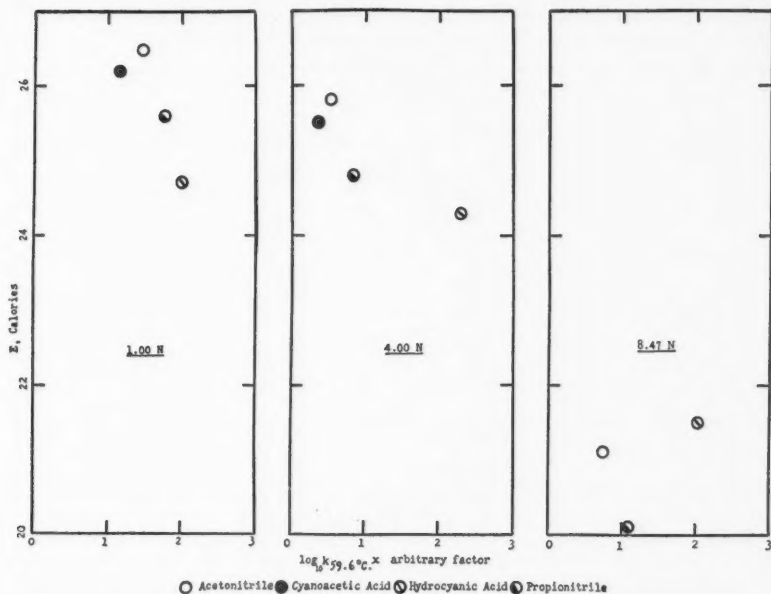
FIG. 1.   *Plot of* $\log_{10} k_{59 \cdot 60 C.}$ *against E for different nitriles, at three acid concentrations.*

and structure difficult (2, pp. 120, 123).   This is immediately apparent from the fact that the rate of propionitrile hydrolysis is intermediate between that of hydrocyanic acid and that of acetonitrile.   It has been suggested (8) that the rate of nitrile hydrolysis increases with the basylous nature of the group on the cyano function.   In this respect, an investigation of meta and para substituted benzonitriles, for which the A factor would probably be constant (2, pp. 120, 123), would be of interest.

The rapid increase in rate of nitrile hydrolysis with acid concentration has been interpreted in several ways.   Results on the hydrolysis of cyanamide in nitric acid solutions of concentration up to 5 N have been expressed by a relation analogous to that for the primary exponential salt effect (1), and have also been related to the acidity function, H_0, of Hammett (3).   Molecular catalysis has also been postulated (9), and, for hydrocyanic acid, a linear relation was obtained between log k and the logarithm of the activity of undissociated hydrochloric acid molecules.

The hydrolysis of acetonitrile in hydrochloric acid of concentrations up to 4 to 5 N displays accordance with all these relations.   This may be seen from

Fig. 2, where rate is plotted against normality, H_0, and log $a^2_\pm$. The slope of the log k vs. H_0 line is 0.92. At higher concentrations where the variation in E is also most marked, deviations from these relations appear. There is at present no theoretical basis for the interrelation of primary salt effect, acidity
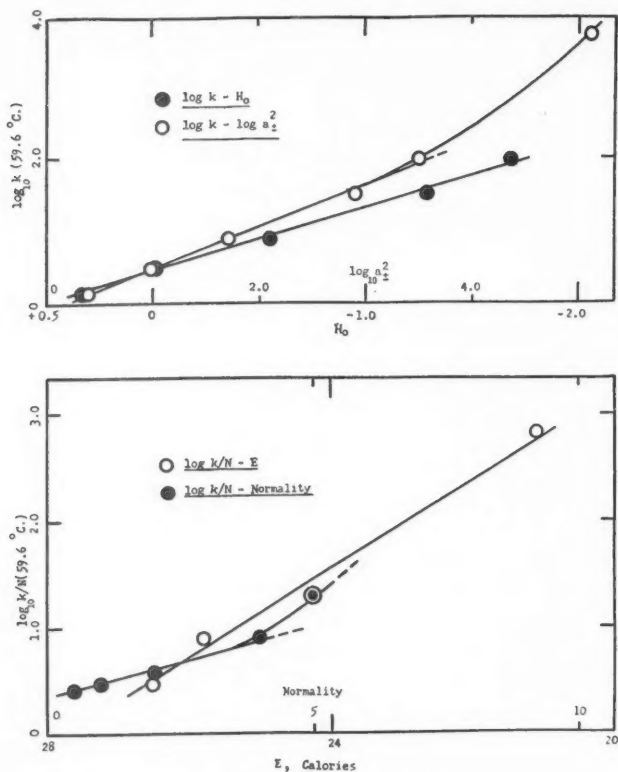

FIG. 2. *Plots of some rate relations.*

function, and log $a^2_\pm$, and some of these relations are probably fortuitous. It would appear, moreover, that the fundamental character of all the relations found here is open to doubt, in view of the simultaneous variations of the Arrhenius constants (Table III). Such variations, as discussed in an earlier paper (16), appear to be real. The plot of log k/N against E, for acetonitrile, is a line, the slope of which is of proper order, although shifted in the direction indicated by the simultaneous variation in A (Fig. 2). This behaviour is similar to that observed with propionitrile where the rate increase also followed the decrease in activation energy.

With hydrocyanic acid, the rate increase with acid concentration is faster than that for the other nitriles. The decrease in E is smaller than that for

acetonitrile or propionitrile, but a simultaneous increase in the A term occurs. This opposing variation of A and E is contrary to the behaviour generally found but accounts in this case for the observed behaviour.   The Hammett relation does not hold for hydrocyanic acid.   In view of the observed changes with acid concentration in E and A for hydrocyanic acid hydrolysis, remarks similar to those made for acetonitrile apply to the relation between rate and square of the mean ion activity, noted particularly for this nitrile.

Explanation of the variation of activation energy with acid concentration observed in the nitrile and amide hydrolysis reactions encounters difficulties. These arise not only from the complexity of highly concentrated solutions of electrolytes, but also because bimolecular reactions involving complex organic molecules, particularly where the solvent participates in the reaction, are most difficult of treatment (14).   The dependence of the activation energy of ionic reactions on dielectric constant, ionic strength, and composition of the solution has been pointed out (17).   Although of lesser importance for reactions involving neutral molecules, these aspects should be considered.   This is very difficult in studies of the variation of rate with acid concentration in concentrated acid media, as simultaneous variations in composition and dielectric constant occur and information concerning the variation of dielectric constant with composition is not obtainable.

The variation of activation energy found in the present studies of nitriles is in the same direction as that found by Leininger and Kilpatrick for the hydrolysis of sucrose (10) and ethylal (11) in concentrated hydrochloric acid. They have explained this variation in terms of a variation of the heat content of the activated intermediate with acid concentration, which is determined principally by the heat of dilution of the oxonium ion, H_3O^+.   In this way, it is possible to account for an activation energy decrease with increase in acid concentration.   This explanation appears analogous to an earlier conclusion (14) that the Arrhenius parameters depend on the internal volume change for the reaction and particularly on the relative solvation of reactants and complex, which presumably depends on concentration.

The change in E with acid concentration is specific for a variety of substrates and a given acid catalyst, or for a variety of catalysts and a given substrate. This would denote in each instance a variable influence on the activation energy of the heats of dilution of substrate and complex.

It appears that there may be a relation between the acidity function, H_0, and the variation of activation energy with acid concentration exhibited by some acid catalyzed reactions.   Of the three reactions cited by Hammett and Paul (3), the rates of which demonstrate the H_0 relation, hydrolysis of sucrose, cyanamide, and ethyl acetate, the hydrolysis of sucrose shows dependence of E on concentration.   Also, since the hydrolysis of nitriles exhibits both Hammett's relation and dependence of E on concentration, the hydrolysis of cyanamide may well display the same behaviour.   The activation energy for the acid hydrolysis of ethyl acetate in highly concentrated solutions

does not appear to have been examined.   However, data reported for hydro-
chloric acid solutions below $0.4 N$ (19) give an indication that in concentrated
solutions there may be an activation energy decrease with increasing acid
concentration.

A parallelism between the H_0 relation and E change may be explained by
variation of solvation of the oxonium ion with change of acid concentration.
As previously mentioned, decrease of solvation could account for the decrease
of activation energy with increasing acid concentration.   At the same time,
removal of an envelope of water molecules from the oxonium ion would
increase the proton transfer tendency, of which H_0 is a measure.

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